Soil carbon balances and stock changes under different cropping and management systems

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Preface

This thesis is submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy (PhD) at Faculty of Science and Technology, Aarhus University, Denmark.

This thesis is the outcome of the PhD research work carried out from October 2014 to January 2018 at the Department of Agroecology, Faculty of Science and Technology, Aarhus University, Denmark. The work was part of the RowCrop project supported by GUDP under the Danish Ministry of Environment and Food, and partly supported by the China Scholarship Council (CSC). The research was supervised by Professor Jørgen E. Olesen, Senior Scientist Peter Sørensen and Professor Bent T. Christensen. The thesis is mainly based on the work presented in the following papers:

Paper I:
Hu, T., Sørensen, P., Wahlström, E.M., Chirinda, N., Sharif, B., Li, X., Olesen, J.E., 2018. **Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass**. Agriculture Ecosystem & Environment 251, 141-148.

Paper II:
Hu, T., Sørensen, P., Olesen, J.E., **Soil carbon varies between different organic and conventional management schemes in arable agriculture**. European Journal of Agronomy 94, 79 -88.

Paper III:
Hu, T., Olesen, J.E., Christensen, B.T., Sørensen, P., **Release of C and N from fodder radish (Raphanus sativus) shoots and roots incubated in soils with different management history**. Soil Science Society of America Journal (submitted).

Paper IV:
Hu, T., Taghizadeh-Toosi, A., Olesen, J.E., Jensen, M.L., Sørensen, P., Christensen, B.T., **Converting temperate long-term arable soil into semi-natural grassland: decadal-scale changes in topsoil C, N, ^{13}C and ^{15}N contents**. European Journal of Soil Science (In preparation).

Abstract

Soil is one of the most important terrestrial carbon (C) stores. In agroecosystems, the amount of soil C in topsoil is variable under different environments and managements. Thus, to increase C input and enhance the soil organic C (SOC) content in cropping systems it is necessary to explore the managements practices that may achieve this, such as using perennial green manure, catch crops and animal manure in cropping systems or even converting arable land to semi-natural grassland.

Published and unpublished data of shoot and root biomass were collected to find the best methods of root biomass estimation. The results were used in the long-term organic and conventional arable crop rotation experiments at Foulum, Jyndevad and Flakkebjerg to estimate C input from different organic materials, where data on aboveground biomass (from green manure, catch crops and crops) and C input from animal manures have been collected every year at each location since 1997. This was compared with C in soil sampled with 4-8 year intervals. In addition, soil samples in organic and conventional treatments, with or without catch crops, were collected from the rotations at Foulum in 2015 for an incubation experiment. Shoot and root material from fodder radish (*Raphanus sativus oleiformis* L.) was separately mixed with the soil samples and incubated for 180 days at 20 °C. To better understand the impacts of conversion from arable land to semi-natural grassland on soil C, the content and isotopic signature of C was measured using soil samples for the years 1942-2012 from the soil archive for the long-term fertiliser experiment at Sandmarken, Askov, where past treatments were: unmanured (O), animal manured (AM) and mineral fertilized (NPK). At Askov, the experiment with crop rotations started in 1894 and were converted to semi-natural grassland in 1998. Correspondingly, published and unpublished data on plant isotopic signatures in crop rotations and semi-natural grassland were collected to enhance the understanding of C and nitrogen (N) turnover in the grassland phase of the experiment.

Results showed that the use of fixed root biomass based on the most influential factors (farming systems and species) provided the lowest error of prediction for estimation of root biomass. For the long-term crop rotation experiments, in terms of the collected root biomass data and the fixed root biomass estimation, using green manure, catch crops and animal manure increased C input by 0.9, 1.0 and 0.7 Mg C ha^{-1} yr^{-1}, respectively, for the years 1997-2004. For SOC, the corresponding figures were 0.4, 0.2 and 0.1 (insignificant) Mg C ha^{-1} yr^{-1}. Since 2005, the advantages for SOC of using green manure disappeared when removing its aboveground part, even though there were still slight advantages for C input. During this

period, there was a 0.7 Mg C ha^{-1} yr^{-1} greater C input when using catch crops, although they did not significantly affect SOC.

During the first four years after converting unfertilised organic treatments into conventional treatments (2005-2008), organic farming with animal manure resulted in a 0.3 Mg C ha^{-1} yr^{-1} larger C input across all sites, but a 0.4 Mg C ha^{-1} yr^{-1} higher SOC loss than conventional farming. In contrast, from 2005 to 2012 at Foulum, organic farming resulted in a 0.7 Mg C ha^{-1} yr^{-1} larger input, and it retained 0.4 Mg C ha^{-1} yr^{-1} more SOC than conventional farming.

Regression analyses for the long-term rotation experiments showed that a similar proportion of above- and belowground plant materials contributed to SOC. The 180-day incubation of fodder radish shoots and roots indicated no differences in recalcitrance between shoots and roots, but more mineral N from shoots was released. Soil management histories did not significantly affect the decomposition of C in plant residues, while the mineralisation of N from plant residues was significantly enhanced in the soils cultivated with catch crops.

Results from Askov showed that during the semi-natural grassland stage, soil C and N started to increase by, respectively, 0.29 Mg C ha^{-1} yr^{-1} and 17 kg N ha^{-1} yr^{-1}, and simultaneously $\delta^{13}C$ and $\delta^{15}N$ values individually dropped by 0.063 ‰ and 0.074 ‰ per year, while the temporal changes did not differ significantly between treatments. The relative ranking of soil C and N levels was O < NPK < AM during both the cultivated stage and the semi-natural grassland stage. Modelling estimated the C input as around 3-4 Mg C ha^{-1} yr^{-1}, indicating higher C input during the semi-natural grassland stage than during the cultivated stage.

In conclusion, the present analysis indicates that root biomass in cereals, catch crops and weeds can be reliably estimated using fixed values based on plant types/species and farming systems. Moreover, below- and aboveground plant C contribute similar proportions of added C to SOC in the long term. In arable systems, the use of perennial green manure (without removing the aboveground part) is better at retaining SOC. Organic farming has a higher C input than conventional farming, but it is uncertain if organic farming enhances SOC. Previous soil management did not affect C decomposition in plant residues, while there was more N released from shoot material and in soils cultivated with a catch crops. Moreover, converting arable land to semi-natural grassland increased soil C and N contents. However, cultivation histories did not show significant impacts on the temporal change of soil C and N.

Sammendrag (Summary in Danish)

Jord udgør en af de vigtigste terrestriske kulstof (C) lagre. I agroøkosystemer varierer mængden af kulstof i jord betydeligt afhængig af klima, jord og dyrkningsform. For at øge kulstoftilførsel og forbedre jordens organiske C (SOC) indhold i dyrkningssystemer er det derfor nødvendigt at undersøge hvordan dyrkningpraksis kan fremme dette, f.eks. ved anvendelse af flerårig grøngødning, efterafgrøder og husdyrgødning i dyrkningssystemer eller endog ved braklægning af dyrkningsjord.

Offentliggjorte og upublicerede data om skud- og rodbiomasse blev indsamlet for at finde de bedste metoder til estimering af rodbiomasse. Resultaterne blev brugt i de langvarige forsøg med økologiske og konventionelle dyrkningssystemer ved Foulum, Jyndevad og Flakkebjerg til estimering af organisk kulstof-input, hvor data på overjordisk biomasse (fra grøngødning, fangstafgrøder og afgrøder) og kulstof i husdyrgødning er blevet indsamlet hvert år på hvert sted siden 1997. Dette blev sammenlignet med kulstofindhold i jord indsamlet med 4-8 års intervaller. Derudover blev jordprøver i organiske og konventionelle behandlinger med eller uden fangstafgrøder indsamlet fra rotationerne hos Foulum i 2015 til et inkubationsforsøg. Skud og rodmateriale fra foderræddike (*Raphanus sativus oleiformis* L.) blev blandet separat med jordprøverne og inkuberet i 180 dage ved 20 °C. For bedre at forstå virkningerne af braklægning af dyrkningsjord på kulstof i jord blev jordens indhold og isotopsammensætningen af C målt ved hjælp i jordprøver for årene 1942-2012 fra jordarkivet fra det langvarige gødningsforsøg på Sandmarken, Askov, hvor tidligere gødningsbehandlinger var: ugødet (O), husdyrgødet (AM) og handelsgødet (NPK). Ved Askov startede dyrkningsforsøget i 1894 og blev omdannet til grønbrak med græs i 1998. Tilsvarende blev offentliggjorte og upubliserede data for kulstof- og kvælstofisotoper i sædskifte- og græs indsamlet for at øge forståelsen af C og kvælstof (N) omsætning i græsfasen af forsøget.

Resultaterne viste, at anvendelsen af en fast værdi for rodbiomasse baseret på de mest indflydelsesrige faktorer (dyrkningssystem og afgrøde) gav den laveste fejl ved prædiktion af rodbiomasse. For de langvarige dyrkningssystemforsøg blev der estimeret kulstofinput på 0,9, 1,0 og 0,7 Mg C ha^{-1} år^{-1} fra anvendelse af henholdsvis grøngødning, efterafgrøder og husdyrgødning for årene 1997-2004. For ændring i SOC var de tilsvarende tal 0,4, 0,2 og 0,1 (ikke signifikant) Mg C ha^{-1} år^{-1}. Efter 2005 forsvandt effekten af grøngødning på øget SOC, formentlig fordi den overjordiske biomasse i grøngødningen blev fjernet i behandlingerne med gødning. I denne periode var der og en øget 0,7 Mg C ha^{-1} år^{-1} C-input fra efterafgrøder, selvom de ikke signifikant påvirkede SOC.

I de første fire år efter konvertering af ugødede økologiske dyrkningssystemer til konventionel dyrkning (2005-2008) resulterede økologisk dyrkning med husdyrgødning i en 0,3 Mg C ha^{-1} år^{-1} større C-input på tværs af alle steder, men et 0,4 Mg C ha^{-1} år^{-1} større SOC-tab end for konventionel dyrkning. I modsætning hertil resulterede økologisk landbrug fra 2005 til 2012 på Foulum i et 0,7 Mg C ha^{-1} år^{-1} større C-input, og 0,4 Mg C ha^{-1} år^{-1} mere SOC end for konventionelt dyrkning.

Regressionsanalyser for de langvarige dyrknigsforsøg viste, at en tilsvarende andel af over- og underjordiske planterester bidrog til SOC. Inkubering af skud og rødder fra roderradise over 180 dage inkubation viste ingen forskelle i nedbrydning af mellem skud og rødder, men mere mineralsk N blev frigivet fra skud end fra rødder. Dyrkningshistorien påvirekse ikke nedbrydningen af det tilførte kulstof i planterester, mens mineraliseringen af N fra planterester blev signifikant forøget ved inkubering i jord med efterafgrøder i dyrkningshistorien.

Resultaterne fra Askov viste, at jordens C og N efter braklægning begyndte at stige med henholdsvis 0,29 Mg C ha^{-1} år^{-1} og 17 kg N ha^{-1} år^{-1}, og samtidig faldt δ^{13}C og δ^{15}N med 0,063 ‰ og 0,074 ‰ om året, mens de tidsmæssige ændringer ikke adskildte sig væsentligt mellem behandlingerne. Den relative rangering af C og N niveau i jord var O <NPK <AM under både dyrkningen og grønbrakken. Anvendelse af en kulstofmodel gave et estimat af C-input på 3-4 Mg C ha^{-1} år^{-1}, hvilket indikerer et øget C-input for grønbrak sammenlignet med landbrugsmæssig dyrkning.

Sammenfattende viser den foreliggende analyse, at rodbiomasse i korn, efterafgrøder og ukrudt kan estimeres pålideligt ved hjælp af faste værdier baseret på plantearter og landbrugssystemer. Desuden bidrager kulstof i under- og overjordiske planterester med tilsvarende andel af tilført kulstof til SOC på langt sigt. I dyrkningssystemer er brugen af flerårig grøngødning (uden at fjerne overjordisk biomasse) bedre til at vedligeholde SOC. Økologisk dyrkning havde et højere C-input end konventionelt landbrug, men det er usikkert, om økologisk dyrkning øger SOC. Tidligere dyrkningspraksis påvirker ikke nedbrydning af C i planterester, mens der var mere N frigivet fra planterester i jord dyrket med efterafgrøder. Endvidere blev jordens C og N-indhold øget ved konvertering fra landbrugsmæssig dyrkning til grønbrak. Dyrkningshistorien havde dog ikke signifikante effekt på ændringen af C og N i jorden.

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List of abbreviations

ADF	Acid detergent fibre
AM	Animal manure/animal manured
CC	Catch crops
LB	Legume based catch crops
LOOCV	Leave one out cross validation
M	Manure
MBE	Mean bias error
NDF	Neutral detergent fibre
NPK	Mineral fertiliser
O	Unmanured
RMSE	Root mean squared error
SOC	Soil organic carbon

1 General introduction

1.1 Soil organic carbon

Globally, soil is one of the most important terrestrial carbon (C) stocks being two or three times the size of atmospheric C, and a small change in soil C can trigger changes in atmospheric greenhouse gas concentrations (Davidson et al., 2000; Lal, 2008; Lehmann and Kleber, 2015). Moreover, soil organic C (SOC) reflects the soil quality and fertility for plant growth (Susanne and Michelle, 1998; Al-Kaisi et al., 2005; Huang et al., 2007; Merante et al., 2017). Properly managed SOC could bring environmental benefits like enhancing soil hydraulic properties, thus contributing to mitigate negative impacts from droughts and extreme rainfall (Blanco-Canqui and Lal, 2004; Gomiero et al., 2011). Storing more C in soil facilitates the improvement of soil structure, and thus leads not only to an enhancement of the soil C level, but also in some cases to reduced of N_2O emissions (Mutegi et al., 2010; Powlson et al., 2011). SOC is a component of soil organic matter, which contains many different nutrients, such as nitrogen (N), phosphorus (P) and sulphur (S) (Kirkby et al., 2011). These nutrients support the growth of plants, and in organic farming SOC in soil organic matter is especially important for soil fertility (Watson et al., 2002; Gomiero et al., 2011; Reganold and Wachter, 2016).

The SOC content is the result of the balance between C input and C loss in soil. In agroecosystems, soil C contents are changeable under different environments and management combinations (Janzen et al., 1997; West and Post, 2002; Crowther et al., 2016). Therefore, C input directly or indirectly from plant materials is the primary management tool to enhance total SOC storage, i.e. through more crop residues and animal manure (Powlson et al., 2011). The effects of other measures, for example tillage and applying mineral fertilisers, on SOC are uncertain (Powlson et al., 2011), where they on the one hand are positive for the growth of crops and thus may increase crop productivity and increase C input in residues, but are also able to accelerate the decomposition of labile C in soil (Chen et al., 2014; Yu et al., 2017). Consequently, C input is the key starting point to SOC stock enhancement, although many organic materials, which may remain in soil for a longer time, are also under consideration (Dungait et al., 2012).

1.2 Organic farming

Organic farming is considered an environmentally friendly agricultural practice for enhancing SOC stock and nutrient cycling (Reganold and Wachter, 2016). Many studies report that topsoil SOC stock is significantly higher in organic farming than in conventional farming (Gomiero et al., 2011; Gattinger et al., 2012; Tuomisto et al., 2012). There is correspondingly a larger total C input in organic farming than conventional farming (Gattinger et al., 2013). The larger total C inputs are generally ascribed to a larger input from external sources, i.e. from manure, slurry and compost. However, organic practices commonly produce lower crop yields than conventional farming (Connor, 2008; Leifeld, 2012; Seufert et al., 2012), and thus organic farming may be assumed in similar farming systems to transfer less C through photosynthesis to the soil (Leifeld et al., 2013). Besides the higher C input, organic farming is also reported to cause less soil erosion, which may further preserve SOC (Reganold and Wachter, 2016).

1.3 Perennial green manure, catch crops and animal manure

Perennial legume-based or non-legume-based grassland and catch crops and application of animal manure are commonly used measures in organic farming for better nutrient utilisation and providing additional C inputs besides crop residues (Olesen et al., 2007; Poeplau and Don, 2015). Perennial grasses have a far larger root biomass than annual crops (Bolinder et al., 2002; Attard et al., 2016), and perennial grassland decreases the artificial disturbance of the soil all year round. Generally, grasslands increase 0.3-1.9 Mg C ha^{-1} yr^{-1} in the SOC pool (Christensen et al., 2009; Müller-Stöver et al., 2012; Rosenzweig et al., 2016). Catch crops grow in the period between main crops, replacing bare fallow during winter, and are able to take up nutrients left by the crops and provide extra C input and protection to the soil, leading to an average annual increase in SOC of 0.3 Mg C ha^{-1} yr^{-1} (Poeplau and Don, 2015). Animal manure is a key fertiliser in organic farming and increases C input through both the enhancement of crop productivity and C input from manure itself. The positive impacts of using animal manure for SOC are widely reported, especially relating to cattle manure (Maillard and Angers, 2014; Zavattaro et al., 2017), but it is necessary to examine such effects locally, since effects can vary depending on local conditions (Maillard and Angers, 2014; Poeplau and Don, 2015).

1.4 Root biomass

Generally, most C inputs originate from plants through photosynthesis, and the C contents in plant materials are estimated from their biomass by multiplying a specific coefficient for the C content in biomass, i.e. 0.45 (Chirinda et al., 2012; Kätterer et al., 2011). Therefore, the C content of plant materials can be calculated if the dry biomass of plants is weighed. Some of the photosynthesised C is stored in plant shoots and some in the root parts, but unlike the aboveground biomass, which can be directly harvested and measured, roots are deeply distributed in soil and thus poorly quantifiable under field conditions (Smucker, 1984; Taylor, 1986). Root biomass is therefore commonly estimated using an allometric method, assuming a certain proportion of plant biomass is allocated to root as root biomass (Johnson et al., 2006; Kätterer et al., 2011; Berti et al., 2016). To enhance the reliability of estimation, plant species and the environmental growing conditions are taken into account when choosing the proper allometric ratios (Campbell et al., 2000; Bolinder et al., 2007). This is because the partitioning can be very sensitive to environmental conditions (Hodge et al., 2000; Muñoz-Romero et al., 2009), whereby, for example, a nutrient application will increase shoot biomass, but root biomass will change very little (Jenkinson, 1981; Anderson, 1988; Huggins and Fuchs, 1997). Recent studies show that species and management practice rather than shoot biomass influence root biomass (Chirinda et al., 2012; Taghizadeh-Toosi et al., 2016). Since organic farming systems lead to a greater root biomass, but conventional farming systems to greater shoot biomass (Chirinda et al., 2012), the use of fixed allometric ratios between shoots and roots may lead to a large bias in root biomass estimation. To enhance the estimation of root biomass, it is therefore necessary to examine the bias of different estimation methods.

1.5 Decomposition of shoots and roots

Based on long-term experiments, Berti et al. (2016) reported that the quality was more important than the quantity of organic inputs, and that the organic input from roots was more recalcitrant than that from shoots. A similar phenomenon has been reported in many other studies based on long-term experiments ranging from 6-50 years, where the humification coefficient of root-sourced C was 2-3 times higher than the C from shoots (Barber, 1979; Plénet et al., 1993; Kätterer et al., 2011). Rasse et al. (2005) found that shoot-derived organic materials decomposed 2.4 times faster than root-derived materials because root materials had better protection from chemical, physicochemical and physical properties. These aforementioned results strongly suggested that, there were more root-derived materials in SOC than shoot-derived materials.

However, shoots and roots may contribute equally to SOC in the long term. Dungait et al. (2012) reported in a review that organic materials did not differ in their long-term decomposition rates as long as they were equally accessible to microbes. Observations in Gentile et al. (2011) in both incubation and field experiments showed the recalcitrance of roots only in the short term, with equal decomposition rates for shoots and roots in the long term. Results from Helfrich et al. (2008) on maize and Austin et al. (2017) on catch crops both support faster decomposition rates of shoots in the short term and equal decomposition rates in the long term. Further experiments on decomposition rates between shoot and root materials are thus necessary to shed more light on these contradictory results.

1.6 Impacts of management histories on soil decomposition abilities

Soil microbial stucture may vary under different management strategies (Leite et al., 2010; Tian et al., 2011; Janusauskaite et al., 2013; Zhen et al., 2014), thus probably further affecting the decomposition ability of the organic materials incorporated in soil. Perennial grasses can produce large quantities of root biomass (Bolinder et al., 2002; Attard et al., 2016), and the continuous fresh C inputs will enhance decomposition of organic materials and thus also soil microbial activity (Acharya et al., 2012; Attard et al., 2016; Rosenzweig et al., 2016; Guo et al., 2017).

Besides perennial grasses, catch crops are commonly used in organic farming systems. They take up the unabsorbed nutrients following the main crop and prevent N leaching (Askegaard et al., 2005; Constantin et al., 2010), thus enhancing nutrient recycling (Müller et al., 2006; Olesen et al., 2007; Thorup-Kristensen and Dresbøll, 2010). Many studies report higher soil C and N contents after long-term use of catch crops (Mazzoncini et al., 2011; Tiemann et al., 2015; Mbuthia et al., 2015). The enhanced input of organic materials and nutrients can also boost microbial activity (Chen et al., 2014).

Animal manure contains both C and nutrients. Applying animal manure not only incorporates extra C and nutrients into the soil, but also enhances soil structural stability and soil porosity, thus further improving the microbial living environment (Francis et al., 2010; Wang et al., 2011). Animal-manured soils generally have a higher N content and SOC stock than soils receiving mineral fertilisers (Plaza et al., 2004; Maillard and Angers, 2014; Francioli et al., 2016). Animal-manured soil may therefore also have higher microbial activity, possibly leading to faster decomposition of organic materials.

1.7 Conversion of arable land to semi-natural grassland

As previously mentioned, artificial soil disturbance, such as tillage, is generally abandoned in semi-natural grassland, which protects the soil C from erosion and oxidisation (Powlson et al., 2014; Yu et al., 2017). However, nutrient input from fertilisers also ceases, which affects the biomass production and thus total residue biomass in grassland (Jones et al., 2014), thus influencing the total C input (Powlson et al., 2011). In non-legume-based grassland, the lack of N input is indicative of the very limited N supply from atmospheric deposition (Hertel et al., 2013). The limited nutrient supply and potentially low C input in semi-natural grassland carries a risk of SOC decline, even if grasslands are broadly reported to increase SOC (Christensen et al., 2009; Müller-Stöver et al., 2012; Rosenzweig et al., 2016). SOC changes may also vary because of different nutrient cycling conditions induced by long-term application of different fertilisers, be they mineral fertilisers or animal manure (Plaza et al., 2004; Maillard and Angers, 2014; Francioli et al., 2016). These fertilisers may also change the microbial activities in the soil (Plaza et al., 2004; Francioli et al., 2016), affecting the decomposition of organic materials, thus leading to further uncertainties in the development of soil C contents.

1.8 Aim and objectives

The overall aim of the thesis was to explore and identify practical management systems that enhance the SOC content in cropping systems. It includes effects of using perennial green manure, catch crops and animal manure in cropping systems and converting arable land to semi-natural grassland. The specific objectives were:

1. To explore a robust method to estimate root biomass and thus enhance the precision and accuracy of the estimated C input from belowground biomass (Paper I)
2. To quantify how much the use of perennial green manure, catch crops and animal manure contributes to C input and thus affects the SOC change (Paper II)

3. To quantify the total C input and C input from plants (both above- and belowground residues) in both organic and conventional farming systems, and further to quantify the change of SOC in both farming systems (Paper II)
4. To explore the recalcitrance of plant materials from above- and belowground plant parts (Papers II & III)
5. To explore how the management histories affect the decomposition of organic materials (Paper III)
6. To quantify the change in SOC of a semi-natural grassland on a post-agricultural field and relate this to different management histories prior to establishing the grassland (Paper IV)

1.9 Hypotheses

The following hypotheses were explored in the studies:
1. Shoot and root biomass vary depending on environmental and management factors, and using the fixed amount for root biomass provide the most robust estimate.
2. Perennial green manure, catch crops and animal manure all increase C input in arable cropping systems, and all result in an enhanced SOC content.
3. Organic arable farming systems result in larger C inputs to soil and generally enhance the SOC stock compared with conventional systems.
4. Root materials have a higher humification coefficient than shoot materials.
5. Using catch crops and animal manure leads to an enhanced decomposition ability of soil, resulting in a higher net C and N mineralisation.
6. The SOC content increases if arable land is converted to semi-natural grassland.
7. The management history of an arable soil will after conversion to semi-natural grassland influence the trend of soil C and N storage.

2 Methodologies

2.1 Overview of methods

To achieve the aim of the thesis, published and unpublished data on shoots and roots were collected to test different methods of root biomass estimation. The results were linked to the long-term organic and conventional arable crop experiments at Foulum, Jyndevad and Flakkebjerg to estimate C input from different plants, where measurements of aboveground biomass, C input from animal manures and SOC content have been collected since 1997. In addition, soil samples from the field experiment at Foulum for the different management systems were collected for incubation. Different materials of different qualities (e.g. roots and shoots) were mixed with the soil samples and were incubated for 180 days.

To better understand the impacts of conversion from arable land to semi-natural grassland on soil C, soil C contents were measured on the soil samples from the soil archive for the long-term field experiment at Sandmarken, Askov, for the years 1942-2012 as the natural abundance of the C isotope subjected to three different past treatments: unmanured (O), animal manured (AM) and mineral fertilized (NPK). At Askov, the crop rotations started in 1894 and were converted to semi-natural grassland in 1998. Corresponding published and unpublished plant isotopic signatures for crop rotations and semi-natural grassland were also collected.

2.2 Root biomass estimation

2.2.1 Cereals

Data for winter and spring wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.) were collected from Chirinda et al. (2012), who sampled shoot and root data in both organic and conventional management systems at Foulum that were started in 1997 (Olesen et al., 2000). Further data for winter wheat for 2013 and 2014 were collected from a conventional experiment initiated in 2002 at Foulum (Munkholm et al., 2008; Hansen et al., 2011). In both experiments, shoots were sampled at maturity and roots were sampled at anthesis. Root dry biomass was converted to 25 cm depth from closest root sampling depth using the Michaelis-Menten function for root distribution with depth as in Kätterer et al. (2011)

in southern Sweden for root depth distribution of small-grain cereals using Equation (1) in Paper I.

2.2.2 Catch crops and weeds

Data for fodder radish (*Raphanus sativus* L.), perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), winter vetch (*Vicia villosa* Roth.), winter rape (*Brassica napus* L.), phacelia (*Phacelia tanacetifolia* Benth.), rye (*Secale cereale* L.), oats (*Avena sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), *Malva sylvestris* L., *Agrostemma githago* L. and chicory (*Cichorium intybus* L.) were collected from Mutegi et al. (2011), Chirinda et al. (2012) and Li et al. (2015) for samples taken at Foulum, from Thorup-Kristensen (2001) for samples from Aarslev, and from Wahlström et al. (2015) for Flakkebjerg. As a supplement to the dataset, three types of catch crop combinations and weeds were sampled in December 2014 for shoot and root biomass determined from the aforementioned long-term organic crop roation at Foulum (Olesen et al., 2000): fodder radish + rye, fodder radish + rye + vetch, chicory + perennial ryegrass + red clover + white clover. Root dry biomass was also converted to 25 cm depth from closest root sampling depth using Equation (2) in Paper I for root distribution with depth.

2.2.3 Root biomass estimation methods

The MIXED procedure of SAS (SAS Institute, 1996) was used to find which factors influence crop shoot, root and the allometric ratios (root/shoot, shoot/root, shoot/(shoot+root) and root/(shoot+root)). For cereals, the year, species (wheat or barley), seeding time (spring or autumn), tillage (ploughing or no tillage), farming system (organic or conventional management) and nitrogen fertilisation rate were considered. For catch crops and weeds, the following factors were taken into account: location, catch crops or weeds, legume-based or non-legume-based, undersowing or sowing following harvest of the main crop, and farming system. Backward elimination was used to remove variables that did not contribute significantly based on the Akaike Information Criterion (AIC). The best model was selected according to the lowest AIC and significant effect ($P < 0.05$) of independent variables. Based on the selected models, different approaches (allometric functions and various determining factors) for root biomass estimation were tested by leave one out cross validation (LOOCV) based on mean bias error (MBE) and root mean squared error (RMSE).

2.3 Arable rotations

2.3.1 Experimental design and management systems

To explore the impacts of cropping systems and specific organic management systems on SOC content, aboveground biomass data and SOC data from long-term experiments started in 1997 at Jyndevad, Foulum and Flakkebjerg were collected. The main crops included in the experiment were: spring barley, spring and winter wheat, winter rye, spring oat, winter triticale (*Triticosecale*), lupin (*Lupinus angustifolius* L.), faba bean (*Vicia faba* L.), a mixture of pea (*Pisum sativum* L.) and spring barley, potato (*Solanum tuberosum* L.), grass-clover, mainly including perennial ryegrass, white clover, red clover and lucerne (*Medicago sativa* L.) (Table 1).

In the first and second cycles from 1997 to 2004, the experiments were conducted at all three locations. There were three factors included in a fully factorial design which contained eight treatment combinations: (i) legume-based perennial green manure crops in organic rotations (with: O1 and O2, without O4), (ii) catch crops (with: +CC, without: -CC), and (iii) animal manure (with: +M, without: -M). Treatment O1 was only applied at Jyndevad, while O4 was applied at both Foulum and Flakkebjerg during this period. Green manure was cut 3-4 times and left on the ground in both O1 and O2, except in 1999 at Jyndevad to enable couch grass control (*Agropyrum repens* L.). Three different types of animal manure were applied during this period, with cattle slurry at Jyndevad, pig slurry at Foulum and anaerobically digested slurry at Flakkebjerg. All straw was incorporated into the soil or left on the ground.

In the third cycle from 2005-2009, rotation O1 was replaced by O4 at Jyndevad (Askegaard et al., 2011). Treatments O2-CC-M and O4-CC-M were converted to C4-CC+IF and C4+CC+IF, where green manure was removed and mineral fertilisers and pesticides were used instead (Shah et al., 2017). Cuttings of green manure were removed in +M treatments. During this period, pig slurry was applied at all three locations. All straw was returned to the field as in the first two cycle.

The experiments at Foulum were the only ones to be continued after 2009 in the fourth cycle. Main management practices were the same as in the second cycle. However, cereal straw was removed in C4 treatments from 2010, and straw of spring barley was also removed in O2 from 2011.

Table 1. Structure of the organic (O) and conventional (C) crop rotations at three locations: JY = Jyndevad, FO = Foulum, FL = Flakkebjerg.

Crop rotations	O1			O2			O4			C4		
Cycles	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]
1st cycle	S. barley:ley	50		S. barley:ley	50		S. oat	40	+[5]			
1997-2000	Grass-clover	0		Grass-clover	0		W. wheat	70	+[5]			
	S. wheat	50	+[3]	W. wheat	50	+[3]	W. cereal	70	+[5]			
	Lupin	0	+[4]	Pea/barley	0	+[4]	Pea/barley	0	+[4]			
	S. barley:ley	50		S. barley:ley	50		W. wheat	50	+[4]			
2nd cycle	Grass-clover	0		Grass-clover	0		S. oat	50	+[4]			
2001-2004	S. oat	30	+[3]	W. cereal	50	+[3]	S. barley	50	+[3]			
	Pea/barley	0	+[4]	Lupin	0	+[4]	Lupin	0				
Locations	JY			JY, FO, FL			FO, FL					
3rd cycle	Discontinued			S. barley:ley	60		S. barley	60	+[4]	S. barley	130	+[3]
2005-2009				Grass-clover	0		F. bean	0	+[4]	F. bean	0	+[3]
				Potato	100		Potato	110		Potato	140	
				W. wheat	100	+[4]	W. wheat	110	+[4]	W. wheat	165	+[3]
Locations				JY, FO, FL			JY, FO, FL			JY, FO, FL		
4th cycle				S. barley:ley	60		S. barley	60	+[4]	S. barley	120	+[3]
2010-2012				Lucerne, 1st	0		Hemp	90		Hemp	125	
				Lucerne, 2nd	0		Peas/barley	0	+[4]	Pea/barley	0	+[3]
				S. wheat	100	+[4]	S. wheat	100	+[4]	S. wheat	110	+[3]
				Potato	100	+[4]	Potato	100	+[4]	Potato	140	+[3]
Locations				FO			FO			FO		

[1]M: Manure application target rates in +M treatments. Unit: kg NH_4-N ha^{-1} in 1st and 2nd cycles and kg total-N ha^{-1} in 3rd cycle. Inorganic fertilizer rates are shown as target mineral N in kg N ha^{-1}.
[2]CC: Crops succeeded by catch crops in +CC treatments.
[3]Monocultures or mixtures of non-N_2-fixing catch crop.
[4]Mixtures of N_2-fixing and non-N_2-fixing catch crop.
[5]White clover.

2.3.2 Soil sampling

SOC content at 0-25 cm depth was sampled in each plot in 1996, 2004 and 2008 at all locations and in 2012 at Foulum. SOC contents were measured with a LECO CNS-1000 analyser with an IR detector (LECO Corporation, St. Joseph, MI, USA) and further corrected by subtracting the carbonates contents. Topsoil SOC quantities were calculated from SOC contents by multiplying with the 0-25 cm soil bulk density for each location and the soil volume. At each location, soil density was assumed identical for the different treatments.

2.3.3 C input estimation

C inputs from green manure, catch crops and crops were taken to be 45 % of the biomass dry matter (Chirinda et al., 2012). The C content in animal manure was detected with a LECO CNS-1000 analyser.

Aboveground biomass of green manures was sampled shortly before each cut. Catch crop samples were taken in early November when growth stopped. The aboveground residues of crops were estimated by measuring the aboveground biomass shortly before maturity and subtracting the harvested biomass at maturity.

Belowground biomass from roots was estimated based on farming system (organic and conventional) and species (Hu et al., 2018). Because of the similar species and cutting times used, red clover data from Bolinder et al. (2002) were used to estimate the fixed root biomass of grass-clover-based green manure. Moreover, for the first and second production years of lucerne data from Bolinder et al. (2002) were also used to estimate the fixed root biomass of lucerne-based green manure in respectively the first and second production year. To estimate the root biomass of cereals, catch crops and weeds, data from Hu et al. (2018) were used for both organic and conventional systems, where legume:barley mixtures were considered as barley. Root biomass data of potatoes in both farming systems were estimated as fixed root biomass collected from 25-30 cm depth in Bolinder et al. (2015). Root biomass data for faba bean (Munoz-Romero et al., 2011), lupin (Russell and Fillery, 1996) and hemp (Amaducci et al., 2008) were collected for both farming systems. Root biomass (potatoes not included) was corrected to 0-25 cm by the Michaelis-Menten function in Kätterer et al. (2011), as previously mentioned. Biomass of root exudates was estimated as 0.65 times that of root dry biomass (Bolinder et al., 2007).

2.3.4 Modelling

The contribution of C from plant materials (aboveground plus belowground), animal manure and original SOC content to the SOC content during 1996-2008 at all locations and 1996-2012 at Foulum was estimated with Equations (1) and (2). The parameters in the equations were estimated using the MIXED procedure of SAS (SAS Institute 2008), where the intercept was set as 0, and block effects nested within locations were considered as random. Data in rotation O1 were also used in modelling for these equations:

$$C_t = K_P \times C_{plant} + K_{AM} \times C_{AM} + K_O \times C_o \quad (1)$$

where C_t is the SOC content (Mg C ha^{-1}) at sampling time t (2008 or 2012); C_{plant} is the C input from plant material (Mg C ha^{-1}); C_{AM} is the C input from animal manure (Mg C ha^{-1}); C_o is the SOC content (Mg C ha^{-1}) of soil sampled in the topsoil (0-25 cm) at the initial 1996 sampling; K_P and K_{AM} are coefficients describing the effects of C inputs from plant materials and animal manure, respectively; and K_O defines the effect of original SOC content on C_t at sampling time t.

Effects of aboveground and belowground parts on the SOC content were examined with the following model:

$$C_t = K_A \times C_{aboveground} + K_B \times C_{belowground} + K_{AM} \times C_{AM} + K_O \times C_o \quad (2)$$

where $C_{aboveground}$ is the C input from aboveground residues (Mg C ha^{-1}); $C_{belowgorund}$ is the C input from roots and rhizodeposition (Mg C ha^{-1}); and K_A, K_B and K_{AM} are coefficients describing the effects of the above- and belowground plant C and animal manure inputs, respectively.

In Equations (1) and (2), K_O refers to the proportion of original SOC left in the soil. Thus, the proportion of C lost every year is calculated as:

$$D_C = 1 - K_O{}^{1/n} \quad (3)$$

where D_C is the proportion of SOC decomposed in one year (decomposition rate of original SOC), and n is the span of years.

2.4 Incubation of shoots and roots

2.4.1 Plant materials and soil samples

Fodder radish, which dominated the catch crops (98 % of aboveground biomass was from fodder radish) and was sampled in December 2014 in the long-term cropping system experiment at Foulum, was selected for shoot and root materials for the incubation experiment. Shoot and root samples were ball-milled and analysed for total C and N content, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents (Van Soest et al., 1991). Soil samples were taken after harvest from the plough layer (0-20 cm) of four different field management systems (O4+CC+M, O4-CC+M, C4+CC+IF, C4-CC+IF) run for the previous 9-17 years at Foulum in the long-term field experiment (Olesen et al., 2000), where C4+CC+IF and C4-CC+IF were separately converted from O2-CC-M and O4-CC-M in 2005. Soil total C, N, mineral N content and pH were measured for each management type (Tables 2 and 3, Paper III).

2.4.2 Incubation experiment

Soil for each management type was packed into steel cylinders (100 cm^3), and moistened to pF 2.0 as 16 % of gravimetric soil water content. Oven-dried material of approximately 500 mg was placed in each cylinder, sandwiched in the middle of the original soil core. Unamended cylinders were used as references. Each soil cylinder was randomly distributed in a RESPICOND VI respirometer (A. Nordgren Innovations AB, Bygdev, Sweden) for 180 days under a constant temperature of 20 °C. Net accumulative CO_2-C released from shoots and roots was calculated as the gap between CO_2-C evolved from amended soil cylinders and the corresponding references. After the incubation, the mineral N content in each soil core was measured.

2.5 Conversion of arable land to semi-natural grassland

2.5.1 Field management

The soil at Sandmarken, Askov, is classified as a coarse sand soil. The field was taken into a long-term fertiliser experiment in 1894. A four-course crop rotation of winter crops, root crops, spring crops and legumes was continued until 1998, when the crop rotation was converted to semi-natural grassland (Table 2). The fertiliser strategies for the crop rotations were: unfertilised (O), mineral fertilised (NPK) and animal manure (AM). More detailed information was presented in Christensen et al. (1994). Grasses (*Lolium perenne* L. and *Festuca rubra* L.) were seeded in 1998, and in 2005 the same grasses were re-seeded directly without tillage. After the conversion from arable land to semi-natural grassland, no further fertiliser or manure were applied, but the names of the pre-treatments were kept.

2.5.2 Soil data

Soil samples from the 0-20 cm depth taken during the arable stage (1942, 1953, 1964, 1976, 1985 and 1996) and in the grassland stage (2000, 2004, 2008 and 2012) were used for isotopic analysis of C and N for soil C and N contents and isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$.

2.5.3 Plant data

To better understand the C and N change in soil, the C content, N content, $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of grasses from O and AM at Sandmarken were sampled in autumn 2011 and spring 2012 by Jensen et al. (Unpublished) for isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$.

2.5.4 C-TOOL modelling

To study the rate of soil C and changes to the C pool distribution, we used C-TOOL (Taghizadeh-Toosi et al., 2014a), where data on soil C, and C input from plant materials and animal manure during the arable stage (1923-1997) were considered to estimate the C input for the semi-natural grassland stage (1998-2012).

Table 2. Rotation elements and fertilizers applied to mineral fertilizer (NPK) and animal manure (AM) plots in Askov-LTE, Sandmarken site since 1894.

Period	Rotation elements				AM				NPK		
	Winter crops	Root crops	Spring crops	Legumes	N	P	K	M [e]	N	P	K
					kg nurtrient ha^{-1}			Mg ha^{-1}	kg nurtrient ha^{-1}		
1894-1922	Winter rye	Mangolds and potatoes [a]	Spring oats	Clover/grass mixture	42	13	31	9	40	13	27
1923-1948	Winter rye	Turnips and potatoes [a]	Spring oats	Clover/grass mixture; Lupines [c]	71	16	65	10+4	70	17	70
1949-1972	Winter rye	Turnips or potatoes [b]	Spring oats	Lupines; Peas [d]	93	20	58	10+4	70	18	66
1973-1997	Winter rye	Turnips or potatoes [b]	Spring barley	Peas	98	19	87	25	100	19	87

[a] Plots divided into two subplots for two crops simultaneously
[b] Turnips in every second rotation
[c] Lupines since 1944
[d] Peas since 1968
[e] Anmial manure was of wet weight. From 1894-1922, farmyard manure was applied. From 1923-1972, Farmyard manure+liquid manure was applied. Since 1973, Cattle slurry was applied.

3 Results

3.1 Arable systems

3.1.1 Shoot and root biomass estimation

For cereals, shoot biomass was most influenced by the quantity of N applied and the time of seeding, while root biomass was most influenced by farming system (organic or conventional), species and year (Table 3). For catch crops and weeds, shoot biomass was most influenced by the type of plant (catch crop or weed), while root biomass was most influenced by plant type and farming system (Table 4). Moreover, cereals, catch crops and weeds each had their unique influential factors for the allometric ratio (Tables 3 and 4). Thus, shoot dry biomass was not a good predictor of root dry biomass. Root dry biomass was best reflected by farming system and species/plant type (catch crop or weed) (Tables 3 and 4). Generally, root dry biomass in organic farming was significantly higher than in conventional farming (Table 5, Paper I).

The best estimation of root dry biomass for cereals, catch crops and weeds was achieved using fixed root estimation, having the lowest MBE and RMSE (Tables 3 and 4). For cereals, pooling the data over years or even species did not increase the MBE and RMSE much (Tables 3 and 4). Thus, the most reliable estimation for cereals was using fixed root biomass based on farming system and species, and for catch crops and weeds estimation based on farming system and plant type (catch crop or weed).

Table 3. Factors affecting shoot, root biomass and their allometric ratios, and comparison of methods for estimating root biomass using cross-validation (LOOCV) for cereals, N=26

Target variables	P values for influential factors [a]						LOOCV for root estimation methods based on most influential factors in	
	Year	Species	Seeding time [b]	Farming system	Nitrogen	Tillage	MBE_P $g\ m^{-2}$	$RMSE_P$ $g\ m^{-2}$
Shoot			0.0205		<0.0001			
Shoot of cereal crops		-	0.0205		<0.0001			
							Root estimation by fixed root amount	
Root	0.0154	0.0022		0.0013			0.0	33.3
Root ignoring year	-	0.0347		<0.0001			-0.3	37.6
Root of cereal crops		-		<0.0001			0.0	39.8
Root (Species)	-	0.7850	-	-	-	-	0.0	57.8
Root (Species, seeding time)	-	0.2831	0.2248	-	-	-	0.0	59.7
							Root estimation by root/shoot ratio	
Root/shoot ratio				<0.0001			3.4	42.7
Root/shoot ratio of cereal crops		-		<0.0001			3.4	42.7
Root/shoot ratio (Species)	-	0.3504	-	-	-	-	10.2	78.1
Root/shoot ratio (Species, seeding time)	-	0.7196	0.1702	-	-	-	9.2	77.0
							Root estimation by shoot/root ratio	
Shoot/root ratio	0.0008	0.0023	0.0222	0.0018	<0.0001		2.0	42.6
Shoot/root ratio ignoring year	-			0.0002	0.0005		-3.5	43.4
Shoot/root ratio of cereal crops	0.0090	-		0.0064	0.0001		-1.0	45.5
Shoot/root ratio (Species)	-	0.2242	-	-	-	-	-17.6	75.4
Shoot/root ratio (Species, seeding time)	-	0.7495	0.1099	-	-	-	-15.5	73.4
							Root estimation by shoot/all or root/all ratio	
Shoot/all or root/all ratio	0.0366	0.0047	0.0166	0.0001	0.0155		1.7	38.2
Shoot/all or root/all ratio ignoring year	-			<0.0001	0.0309		0.5	38.5
Shoot/all or root/all ratio of cereal crops		-		<0.0001	0.0309		0.5	38.5
Shoot/all or root/all ratio (Species)	-	0.3239	-	-	-	-	6.3	77.1
Shoot/all or root/all ratio (Species, seeding time)	-	0.7325	0.1616	-	-	-	5.7	75.9

[a] Factors with '-' were not included in the statistical analysis. Blank cells were items included in the statistical analysis. Factors shown in p values were used for leave one out cross validation (LOOCV). Factors in brackets mean the only factors considered for LOOCV.

[b] seeded in spring or autumn.

Table 4. Factors affecting shoot, root biomass and their allometric ratios, and comparison of methods for estimating root biomass of catch crops and weeds using cross-validation (LOOCV)

Target variables	P Value for influential factors [a]					Test for root estimation methods based on most influential factors in LOOCV		
	Location	Farming system	CC or not [b]	LB or not [c]	Undersown or not [d]	MBE_P g m^{-2}	$RMSE_P$ g m^{-2}	N
Shoot			0.0062					
						Root estimation by fixed root amount		
Root		0.0347	0.0009			0.2	43.0	30
Root (CC or not, Farming system)	-	0.0347	0.0009	-	-	0.2	43.0	30
Root (CC or not, LB or not)	-	-	-	0.6430	-	0.0	46.3	30
						Root estimation by root/shoot ratio		
Root/shoot ratio	0.0294	0.0138				20.8	87.8	29
Root/shoot ratio (CC or not, Farming system)	-	0.0885	0.5707	-	-	30.3	90.5	30
Root/shoot ratio (CC or not, LB or not)	-	-	-	0.2133	-	29.3	92.2	30
						Root estimation by shoot/root ratio		
Shoot/root ratio			0.0116			-18.5	67.2	30
Shoot/root ratio (CC or not, Farming system)	-	0.2819	0.0279	-	-	5.7	80.9	30
Shoot/root ratio (CC or not, LB or not)	-	-	-	0.2196	-	-15.9	71.8	30
						Root estimation by shoot/all or root/all ratio		
Shoot/all; root/all ratio						11.7	76.6	30
Shoot/all; root/all ratio (CC or not, Farming system)	-	0.1060	0.9298	-	-	17.2	80.9	30
Shoot/all or root/all ratio (CC or not, LB or not)	-	-	-	0.1796	-	15.5	83.5	30

[a] Factors with '-' were not included in statistical analysis for more influential factors. Blank cells were items included in the statistical analysis. Factors shown in p values were used for leave one out cross validation (LOOCV). Factors in brackets mean the only factors considered for LOOCV.
[b] CC or not means catch crops or weeds.
[c] LB or not means legume based or non-legume based catch crops.
[d] Undersown or not means undersowing or not undersowing catch crops.

3.1.2 C input and SOC change by using green manure, catch crops and animal manure

Green manure

From 1997 to 2004 across all sites, application of green manure increased C input by 0.9 Mg C ha^{-1} yr^{-1} more in O2 than in O4 (Table 5) from both above- and belowground biomass. During 2005-2008 across all sites, the comparison was only conducted for fertilised treatments. Comparing O2 with O4 during 2005-2008 the use of green manure increased the C input by only 0.2 Mg C ha^{-1} yr^{-1}, because the aboveground part of green manure was removed from 2005 (Table 5). From 2005-2012 at Foulum, green manure without aboveground biomass in O2 increased C input by only 0.3 Mg C ha^{-1} yr^{-1} compared with O4 (Table 5).

From 1997 to 2004 across all sites, the application of green manure significantly mitigated the decrease in SOC by 0.40 Mg C ha^{-1} yr^{-1} (Table 6). During 2005-2008, in the fertilised treatments, after removing the aboveground part of green manure, the better SOC retention in O2 compared with O4 disappeared (Table 6). From 2005-2012 in the fertilised treatments at Foulum, O4 retained significantly more SOC than O2.

Catch crops

From 1997 to 2004 across all locations, the use of catch crops resulted in a higher C input than from weeds of about 1.0 Mg C ha^{-1} yr^{-1} (Table 5), mainly attributed to the catch crops, with a smaller contribution from the crops due to fertility enhancing effects of catch crops (Fig. 1a, Paper II). During 2005-2008, catch crops helped build up an extra 0.7 Mg C ha^{-1} yr^{-1} in the fertilised treatments (Table 5), with 0.6 Mg C ha^{-1} yr^{-1} deriving from the catch crops and the rest from the crops. From 2005-2012 at Foulum, catch crops gave a 0.7 Mg C ha^{-1} yr^{-1} higher C input in the fertilised treatments (Table 5), where 0.6 Mg C ha^{-1} yr^{-1} of this derived from the catch crops, and the remainder from the crops.

Across all sites, the use of catch crops thus enhanced SOC by 0.21 Mg C ha^{-1} yr^{-1} during 1997-2004 (Table 6), but there were no significant effects of catch crops at any site after 2005 (Table 6).

Animal manure

From 1997-2004, the use of animal manure enhanced C input by over 0.6 Mg C ha^{-1} yr^{-1} (Table 5) with 0.2 Mg C ha^{-1} yr^{-1} deriving from the animal manure and over 0.4 Mg C ha^{-1} yr^{-1} from the crops (Fig. 1a, Paper II).

During this period, there was an increase in SOC from using animal manure of 0.14 Mg C ha^{-1} yr^{-1}, but this was not significant across all locations. At Jyndevad and Flakkebjerg there were some significant results (Table 6), where cattle slurry and anaerobically digested slurry were applied separately.

3.1.3 C input and SOC change in organic farming vs conventional farming

From 2005, the unfertilised treatments in organic rotations were converted to conventional management using mineral fertilisers and pesticides. The comparison between organic and conventional rotations was conducted using fertilised treatments in O4 and C4.

For 2005-2008, across all locations, C input was 3.9 Mg C ha^{-1} yr^{-1} in O4, where animal manure accounted for 0.3 Mg C ha^{-1} yr^{-1} (Fig. 1b, Paper II). C input in the conventional C4 rotation was 3.6 Mg C ha^{-1} yr^{-1} (Table 5), which was significantly lower than in the organic O4 rotation. For 2005-2012 at Foulum, O4 contributed 4.1 and C4 contributed 3.4 Mg C ha^{-1} yr^{-1} (Table 5), and the C input from animal manure in the organic rotation again was 0.3 Mg C ha^{-1} yr^{-1} (Fig. 1c, Paper II). The C inputs in C4 were also here significantly lower than for the similar O4 crop rotation.

For 2005-2008 across all locations, the change in SOC in O4 was -0.38 Mg C ha^{-1} yr^{-1}, which was a significantly larger decline than the -0.03 Mg C ha^{-1} yr^{-1} in C4 (Table 6). For the longer time span of 2005-2012, on the other hand, the change in SOC in O4 and C4 at Foulum was, respectively, –0.04 and –0.41 Mg C ha^{-1} yr^{-1} (Table 6), where SOC in O4 decreased significantly less than in C4.

Table 5. Mean carbon input (Mg C ha^{-1} yr^{-1}) estimated in different treatments at Jyndevad, Foulum, Flakkebjerg during 1997-2004 (Jyndevad, Foulum and Flakkebjerg), 2005-2008 (Jyndevad, Foulum, Flakkebjerg) and 2005-2012 (Foulum). Analysis was also conducted with data of all three locations combined during 1997-2004 and 2005-2008. Part I of the table shows carbon inputs for individual treatment components and part II shows main effects of individual treatments. In part II treatments with –M were not used in analysis after 2004.

Treatment	Jyndevad		Foulum			Flakkebjerg		All locations	
	1997-2004	2005-2008	1997-2004	2005-2008	2005-2012	1997-2004	2005-2008	1997-2004	2005-2008
Part I									
O2-CC-M	4.43 c	—	5.48 e	—	—	5.57 c	—	5.16 c	—
O2-CC+M	5.33 b	3.62 bc	6.02 cd	4.46 c	4.19 c	6.01 b	3.64 cd	5.79 b	3.91 c
O2+CC-M	4.96 b	4.44 a	6.18 bc	5.41 a	5.48 a	6.13 b	4.45 a	5.76 b	4.77 a
O2+CC+M	5.75 a	4.32 a	6.32 b	4.72 b	4.58 b	6.36 a	4.15 ab	6.14 a	4.39 b
O4-CC-M	—	—	3.93 g	—	—	3.62 e	—	3.48 e	—
O4-CC+M	—	3.30 d	4.77 f	3.63 e	3.50 d	4.60 d	3.11 ef	4.39 d	3.35 e
O4+CC-M	—	3.61 bc	5.80 d	4.20 d	4.17 c	5.34 c	3.38 de	5.27 c	3.73 d
O4+CC+M	—	4.48 a	6.62 a	4.89 b	4.74 b	6.20 ab	4.12 b	6.11 a	4.50 b
C4-CC+IF	—	3.33 cd	—	3.61 e	3.15 e	—	3.01 f	—	3.32 e
C4+CC+IF	—	3.71 b	—	4.06 d	3.66 d	—	3.72 c	—	3.83 cd
Part II									
O2	—	3.97 a	6.00 a	4.59 a	4.39 a	6.02 a	3.90 a	5.71 a	4.15 a
O4	—	3.89 a	5.28 b	4.26 b	4.12 b	4.94 b	3.62 b	4.81 b	3.92 b
C4	—	3.52 b	—	3.84 c	3.41 c	—	3.36 c	—	3.57 c
-CC	4.88 b	3.42 b	5.05 b	3.90 b	3.62 b	4.95 b	3.25 b	4.77 b	3.52 b
+CC	5.36 a	4.17 a	6.23 a	4.55 a	4.33 a	6.01 a	4.00 a	5.76 a	4.24 a
-M	4.70 b	—	5.35 b	—	—	5.17 b	—	4.94 b	—
+M	5.54 a	—	5.93 a	—	—	5.79 a	—	5.59 a	—

For each group (treatments in Part I; rotations, catch crops and manure respectively in Part II) mean values having different letters within a column are significantly different at the 0.05 significance level.

Table 6. Mean change of soil organic carbon content (Mg C ha^{-1} yr^{-1}) in 0-25 cm depth in different treatments at Jyndevad, Foulum, Flakkebjerg during 1997-2004 (Jyndevad, Foulum and Flakkebjerg), 2005-2008 (Jyndevad, Foulum, Flakkebjerg) and 2005-2012 (Foulum). Analysis was also conducted with data of all three locations combined during 1997-2004 and 2005-2008. Part I of the table shows carbon inputs for individual treatment components and part II shows main effects of individual treatments. In part II treatments with –M were not used in analysis after 2004.

Treatment	Jyndevad		Foulum			Flakkebjerg		All locations	
	1997-2004	2005-2008	1997-2004	2005-2008	2005-2012	1997-2004	2005-2008	1997-2004	2005-2008
Part I									
O2-CC-M	0.10^{ab}	—	-0.84^{abc}	—	—	-0.18^{cd}	—	-0.30^{bc}	—
O2-CC+M	0.18^{ab}	-0.33^{ab}	-1.13^{bc}	-0.13^{a}	-0.17^{ab}	0.00^{abc}	-0.25^{abc}	-0.32^{bc}	-0.25^{ab}
O2+CC-M	-0.31^{b}	0.05^{a}	-0.66^{ab}	0.08^{a}	-0.15^{ab}	0.15^{ab}	0.07^{a}	-0.27^{b}	0.06^{a}
O2+CC+M	0.40^{a}	-0.95^{bc}	-0.44^{a}	-0.02^{a}	-0.56^{b}	0.27^{a}	-0.01^{ab}	0.07^{a}	-0.34^{ab}
O4-CC-M	—	—	-1.26^{bc}	—	—	-0.65^{e}	—	-0.80^{d}	—
O4-CC+M	—	-1.02^{bc}	-1.16^{bc}	0.39^{a}	-0.20^{ab}	-0.37^{de}	-0.36^{abc}	-0.61^{cd}	-0.33^{ab}
O4+CC-M	—	-0.53^{abc}	-1.23^{bc}	0.24^{a}	-0.46^{b}	-0.09^{bcd}	-0.61^{c}	-0.51^{bcd}	-0.30^{ab}
O4+CC+M	—	-1.40^{c}	-1.26^{c}	0.58^{a}	0.13^{a}	-0.03^{abc}	-0.50^{bc}	-0.49^{bcd}	-0.44^{b}
C4-CC+IF	—	-0.17^{ab}	—	0.11^{a}	-0.30^{ab}	—	0.19^{a}	—	0.04^{a}
C4+CC+IF	—	-0.81^{abc}	—	0.64^{a}	-0.53^{b}	—	-0.16^{abc}	—	-0.11^{ab}
Part II									
O2	—	-0.64^{ab}	-0.77^{a}	-0.08^{b}	-0.37^{b}	0.06^{a}	-0.14^{ab}	-0.21^{a}	-0.30^{ab}
O4	—	-1.21^{b}	-1.23^{b}	0.48^{a}	-0.04^{a}	-0.28^{b}	-0.43^{b}	-0.61^{b}	-0.38^{b}
C4	—	-0.49^{a}	—	0.38^{ab}	-0.41^{b}	—	0.01^{a}	—	-0.03^{a}
-CC	0.14^{a}	-0.51^{a}	-1.10^{a}	0.12^{a}	-0.22^{a}	-0.30^{b}	-0.14^{a}	-0.51^{b}	-0.18^{a}
+CC	0.04^{a}	-1.05^{a}	-0.90^{a}	0.40^{a}	-0.32^{a}	0.07^{a}	-0.23^{a}	-0.30^{a}	-0.30^{a}
-M	-0.10^{b}	—	-1.00^{a}	—	—	-0.19^{b}	—	-0.48^{a}	—
+M	0.29^{a}	—	-1.00^{a}	—	—	-0.03^{a}	—	-0.34^{a}	—

For each group (treatments in Part I; rotations, catch crops and manure respectively in Part II) mean values having different letters within a column are significantly different at the 0.05 significance level.

3.1.4 Recalcitrance of roots vs shoots

Modelling

When combining the data for all sites from 1997 to 2008, the humification rate of the C input from plant materials in model I was about 12 % (*$p<0.01$*) (Table 5, Paper II). Animal manure contributed about 14 % of the C input (*$p>0.05$*) to the topsoil, but the value was very variable (Table 5, Paper II). As in model II, aboveground and belowground plant materials contributed separately 4 % (*$p>0.05$*) and 19 % (*$p<0.01$*) to the C input (Table 5, Paper II). The AIC and RMSE values were both lower in model I than in model II, indicating a higher confidence with model I, which considered plant materials as a whole (Table 5, Paper II).

During 1997-2012 at Foulum, plant materials contributed about 11 % (*$p<0.01$*) to C input (Table 5, Paper II). The contribution from animal manure was 40 % of C, but very variable (*$p>0.05$*) (Table 5, Paper II). Splitting the C input from plant materials into aboveground and belowground parts showed similar contributions from the two parts at around 10 %, but both became statistically insignificant, and simultaneously AIC and RMSE values increased (Table 5, Paper II). These results indicated that C inputs from aboveground and belowground plant materials should be considered as a whole, and both parts were important for SOC.

Incubation

During the first 10 days, C decomposition from shoots and roots was 43% and 32%, respectively, and the C evolved from shoots was significantly higher than that from roots (Table 4, Paper III). However, in the following 80 days, the opposite trend appeared, where the C evolved from roots was significantly higher than from shoots (Tables 3 and 4, Paper III). This narrowed the gap in cumulative emissions between roots and shoots during the first 10 days of the incubation (Fig. 1). During the last 90 days, there were no significant differences between shoots and roots (Table 3, Paper III). During the entire 180 days of incubation, there were no significant differences in net C mineralisation from applied roots and shoots, and generally 58 % of the C in shoots and roots was mineralised (Table 4, Paper III). In contrast, plant materials showed significant effects on N mineralisation (Table 3, Paper III), where net N mineralised from shoots and roots was 42 % and 28 %, respectively, during the 180 days of incubation (Table 4, Paper III).

3.1.5 Impacts of soil management histories on soil decomposition abilities

Management histories did not influence the net CO_2-C evolution from applied materials at any time during incubation (Table 3, Paper III), but they did have a significant impact on net N mineralisation of applied materials (Table 3, Paper III). This effect could be ascribed to the previous use of catch crops, where soil managed with catch crops showed higher N mineralisation rates of added materials, though not always at statistically significant levels (Table 4, Paper III).

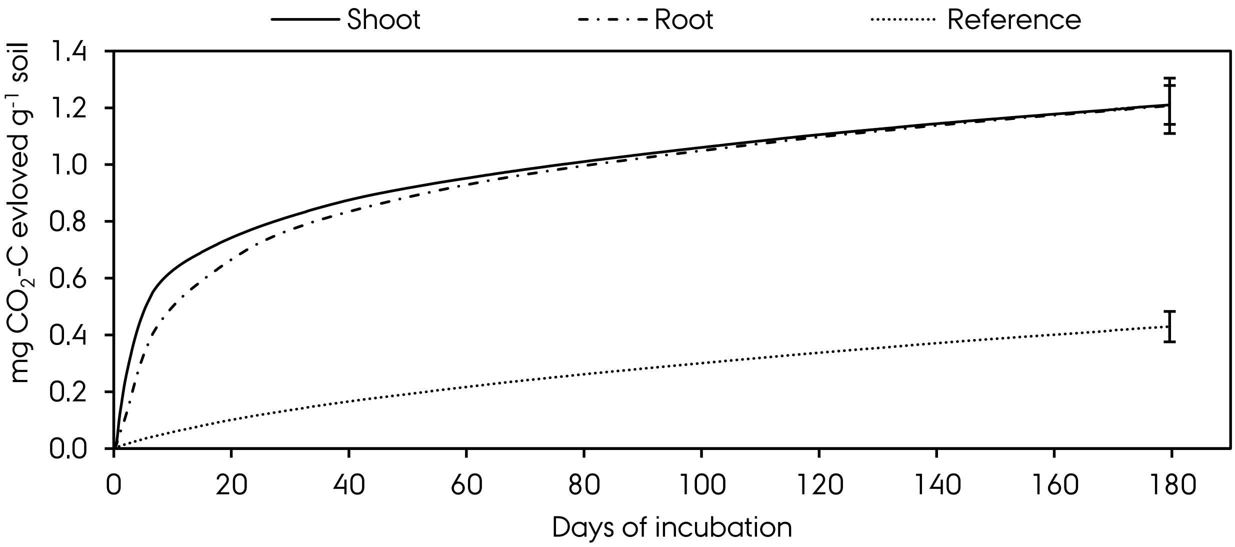

Fig. 1 Cumulated CO_2-C evolution from reference soil with no amendment and soil amended with shoots or roots during the incubation period (180 days). Average of four soils with different management history. Bars indicate standard deviation (n=12).

3.2 Grassland systems

3.2.1 Change of soil C and N contents

In 1942, after nearly 50 years of crop rotations, soil C and N contents in the three treatments were at different levels (Table 7), where O ranked the lowest, NPK in the middle, and AM the highest (Fig. 2a and 2b). During this period, the C content in all treatments decreased at a rate of 0.1 Mg C ha^{-1} yr^{-1} and the N content increased at a rate of 12 kg N ha^{-1} yr^{-1} (Table 7).

In 1998, the arable land was converted to semi-natural grassland with no additional N input, resulting in C and N contents increasing in all treatments (Figs. 2a and 2b) by 0.29 Mg C ha^{-1} yr^{-1} and 17 kg N ha^{-1} yr^{-1} (Table 7). The relative rankings of the different treatments remained as during the arable stage (Fig. 2).

Table 7 Slope of multiple linear regression of C content, N content, δ^{13}C and δ^{15}N on year (± standard error of the mean), where treatment is included as fixed effect. Regressions were performed separately for the arable land period (1942-1996) and the semi-natural grassland period (2000-2012).

Target items	Arable land period (1942-1996)	Semi-natural grassland period (2000-2012)
C (Mg C ha^{-1})	-0.10 ± 0.01	0.29 ± 0.05
N (Mg N ha^{-1})	-0.012 ± 0.002	0.017 ± 0.005
δ^{13}C (‰)	0.0021 ± 0.0007	-0.063 ± 0.005
δ^{15}N (‰)	0.014 ± 0.006	-0.074 ± 0.010

All slopes were statistically significant at the $p < 0.05$ level.
Treatments in all target items were statistically significant at the $p < 0.05$ level.

3.2.2 Change of isotopic signatures of δ^{13}C and δ^{15}N in soil

Before the conversion, values of δ^{13}C and δ^{15}N generally increased by, respectively, 0.002 ‰ and 0.014 ‰ per year (Table 7), with the lowest δ^{13}C and highest δ^{15}N in AM (Figs. 3a and 3b). After the conversion, the relative rankings of δ^{13}C and δ^{15}N in the treatments were as during the arable period (Fig. 3a and 3b), while δ^{13}C and δ^{15}N values dropped at a rate of, respectively, 0.063 ‰ and 0.074 ‰ per year (Table 7).

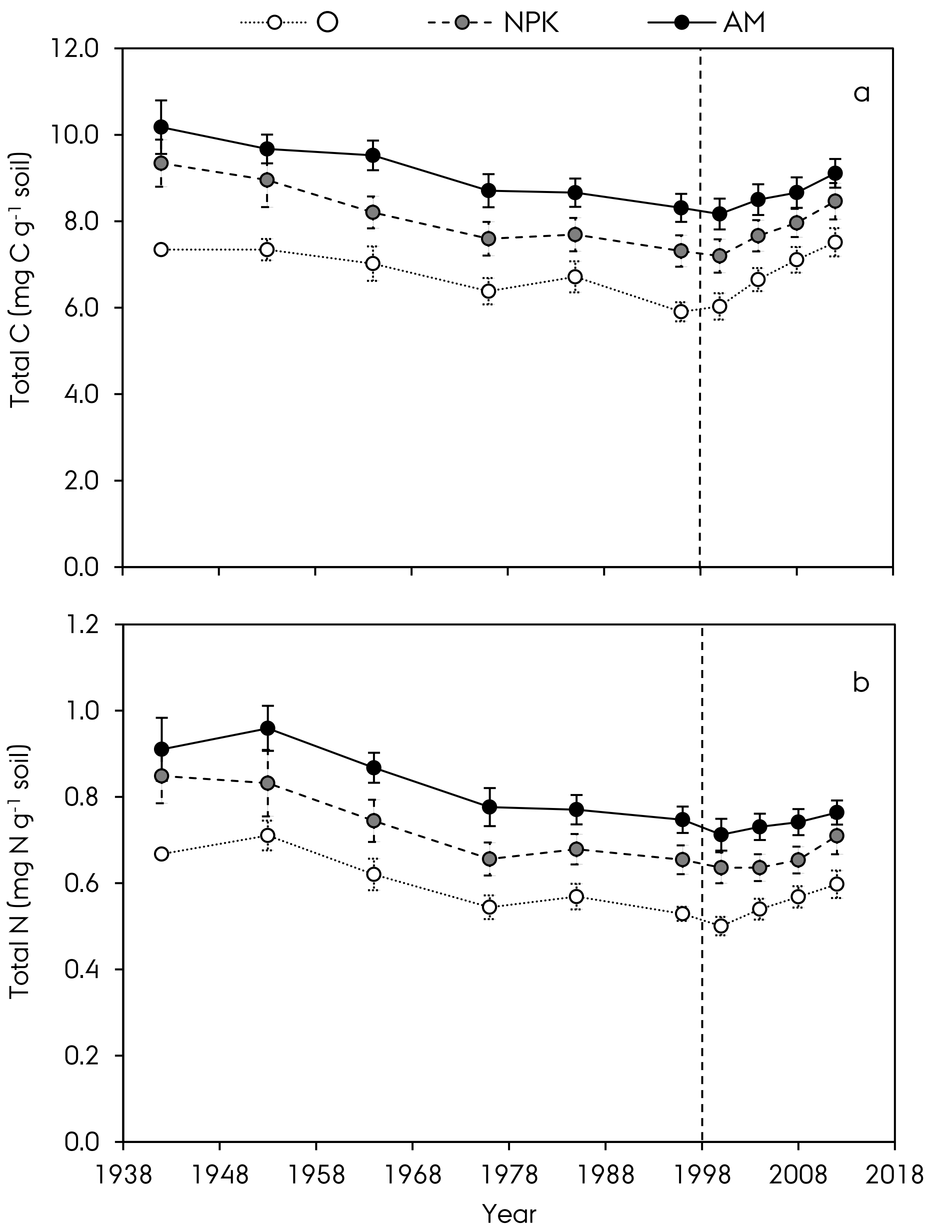

Fig. 2 Temporal changes of C content (a) and N content (b) in 0-20 cm soil. Before year 1998, land was in arable use, while it was converted to semi-natural grassland since then. Points in white, grey and black show Unmanured (O), mineral fertilised (NPK) and animal manured (AM) treatments during arable period, respectively. Bars indicate standard error of the mean.

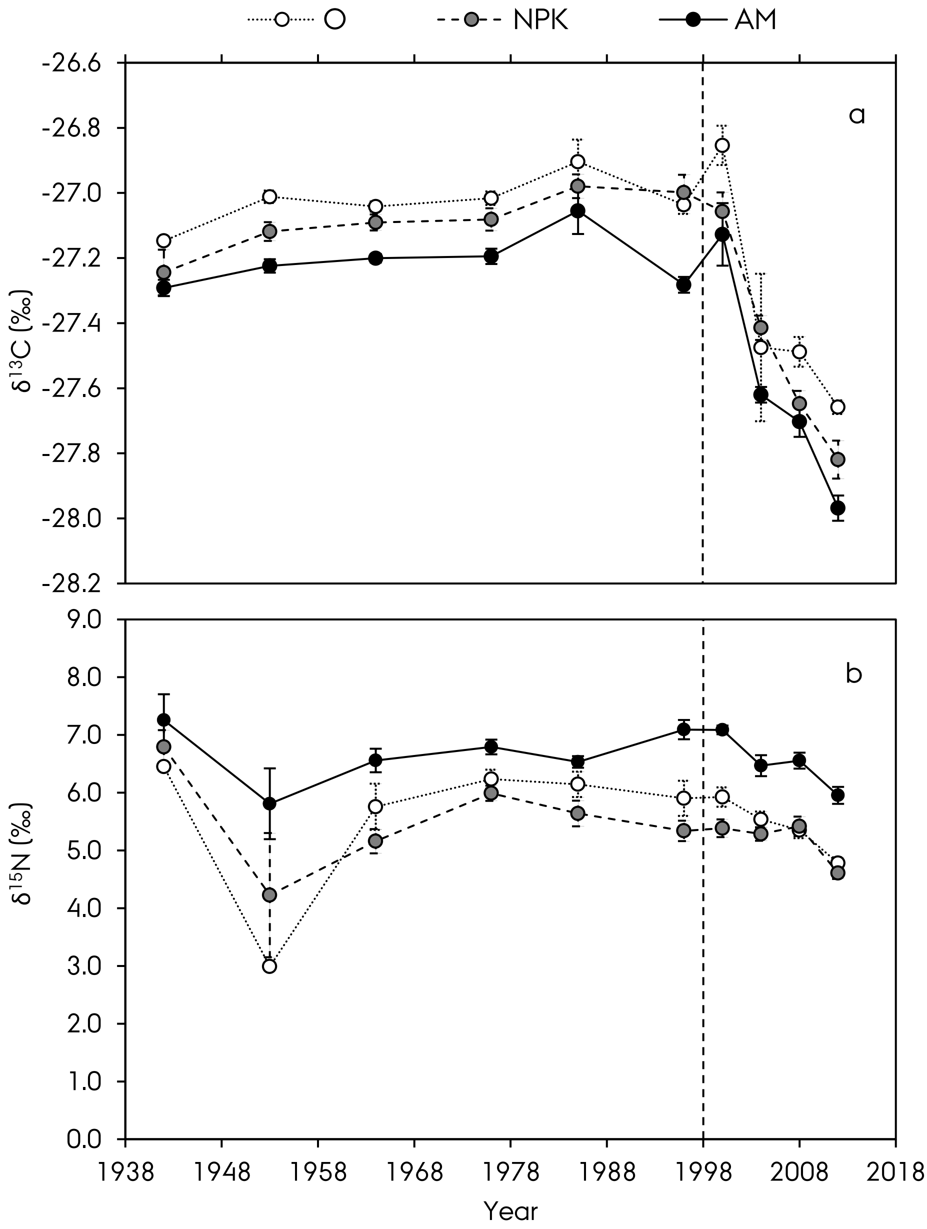

Fig. 3 Temporal changes of $\delta^{13}C$ (a) and $\delta^{15}N$ (b) in 0-20 cm soil. Before year 1998, land was in arable use, while it was converted to semi-natural grassland since then. Points in white, grey and black show Unmanured (O), mineral fertilised (NPK) and animal manured (AM) treatments during arable period, respectively. Bars indicate standard error of the mean.

3.2.3 C and N contents and isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ in grass samples

C content grass samples in O and in AM was very close, around 42-43 % (Table 8). N content in grass from AM was slightly higher than in O, but still very close around 1.5 %. Isotopic signatures of $\delta^{13}C$ was around –29 ‰ in O and close to –30 ‰ in AM, and the difference was significant in autumn. Isotopic signatures of $\delta^{15}N$ in grass from AM was significantly higher than in O, and they were respectively around 0.7 ‰ and -1.4 ‰.

Table 8 C and N isotopic compositions and contents in grasses (± standard error of the mean) sampled in autumn 2011, and in spring 2012 (Jensen et al., Unpublished) from unfertilised (O), and animal manured (AM) at Askov

	$\delta^{13}C$ (‰)	C content (%)	$\delta^{15}N$ (‰)	N content (%)
		Autumn 2011		
O	-29.0 ± 0.2^a	41.7 ± 0.7	-1.3 ± 0.4^b	1.5 ± 0.1
AM	-29.8 ± 0.1^b	42.3 ± 0.6	0.6 ± 0.4^a	1.6 ± 0.1
		Spring 2012		
O	-29.2 ± 0.1	43.4 ± 0.1	-1.5 ± 0.4^b	1.4 ± 0.1^b
AM	-29.4 ± 0.1	43.5 ± 0.1	0.8 ± 0.4^a	1.6 ± 0.1^a

3.2.4 C-TOOL-simulated C input and C pool distribution

Based on the measured topsoil organic C and the C input in topsoil over the arable years, C-TOOL simulated the change in topsoil organic C, and the C input during the grassland stage was optimised to achieve the best fit with observed SOC (Fig. 4). Simulated SOC values generally fitted the measured values well, although there were slightly underestimations in treatments NPK and AM (Fig. 4). Even though the underestimation might reflect too low estimated in C input, the simulated C inputs were similar for the different previous fertilisation treatments and around 3-4 Mg C ha^{-1} yr^{-1} (Fig. 4).

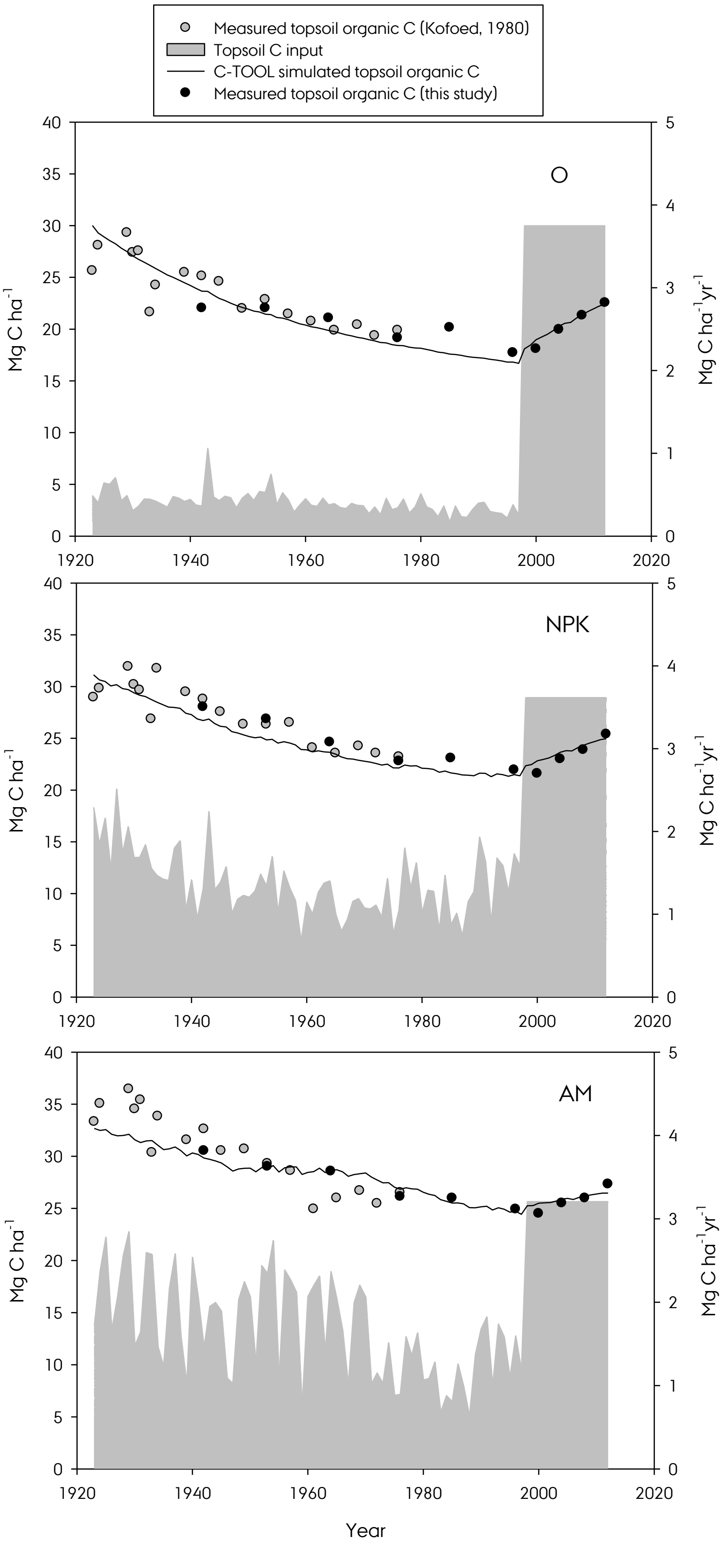

Fig. 4 C-TOOL simulated topsoil (0-20 cm) organic carbon (lines), measured topsoil (0-20 cm) organic C (points, from this study and Kofoed, 1980), and the amount of C input into topsoil over the years (grey bars).

4 Discussion

4.1 Arable system

4.1.1 Root biomass estimation

Shoots and roots cooperate, where shoots provide photosynthesized materials and roots absorb nutrients from soil (Thornley, 1972). The need for belowground resources, like nutrients, stimulates the growth of roots, and the need for photosynthesized material stimulates the growth of shoots (Thornley, 1972; Poorter and Nagel, 2000). During the growing season, the cooperation between plant parts and environmental factors determine the final biomass of shoots and roots. Thus for any given species, it is the weather and the soil conditions that determine how much can be photosynthesized and allocated to shoots or roots.

In our results, fixed root biomass based on year, species or plant types and farming system best estimated root biomass, achieving the lowest MBE and RMSE (Tables 4 and 6, Paper I). The year factor became significant, because of a much lower root biomass for cereals at 0-25 cm depth in 2013, indicating the influence of weather. Table 3 in Paper I shows that precipitation was low in spring that year. However, the precipitation mainly affected the root biomass distribution with depth (Table S1, Paper I), since similar root biomasses were observed for the 0-60 cm depth in 2013 and 2014 (data not shown). Moreover, genotypic variation between species and plant types could cause different specific root-shoot cooperation strategies (Fakhri et al., 1987; Clark et al., 2003), and thus the development of different root biomasses in different species or plant types. In addition, nutrients, especially nitrogen in organic farming, are less available than in the conventional systems, even though the total input is not always less (Stockdale et al., 2002). This lower availability of nutrients is one of the major stimuli for root growth leading to a relatively larger root biomass (Poorter and Nagel, 2000; Lonhienne et al., 2014).

Farming system was the most significant influential factor for roots, but not for shoots (Tables 3 and 4). Thus, the following general observations on the effect of the two farming systems can be made: 1) There was more shoot biomass and general less root biomass in conventional farming, and the opposite in organic farming; 2) there was more total (shoot+root) biomass and general less root biomass in conventional farming, and a relatively equal distribution between shoots and roots in organic farming; 3) there is less difference in root biomass (highest in organic farming) than shoot biomass (highest in conventional farming) between the two farming systems. With the existence of all three relations, estimating root biomass based on shoot biomass would be biased when estimating root biomass of both organically and conventionally grown crops using allometric relations, but root biomass could be reliably estimated using a fixed root biomass based on farming system and plant species, at least in Northern Europe.

4.1.2 C input and SOC change by using green manure, catch crops and animal manure

During the experiments, the use of green manure, catch crops and animal manure all significantly increased total soil C input (Table 5). However, the increased C input did not necessarily enhance measured SOC (Table 6).

Including grassland in crop rotations generally increases SOC (Leifeld, 2012), and this effect ranges from 0.3-1.9 Mg C ha^{-1} yr^{-1} (Christensen et al., 2009; Müller-Stöver et al., 2012; Rosenzweig et al., 2016). The benefit of using green manure for SOC was observed in the treatments where the aboveground green manure was not harvested (Table 6), because the harvest of green manure largely offset the advantage of C input (Table 5). This also indicates that growing a green manure crop for harvesting does not necessarily improve SOC. The benefit of using green manure with retention of aboveground materials could be ascribed to the larger C input, especially from root materials. The green manure only occupied a quarter of the crop rotation period, but enhanced SOC by 0.4 Mg C ha^{-1} yr^{-1}; this corresponds to a potential of 1.6 Mg C ha^{-1} yr^{-1} for each year of grass, which is slightly above the estimation from Taghizadeh-Toosi et al. (2014b) for grass in Denmark by 0.95 Mg C ha^{-1} yr^{-1} in 0-25 cm depth.

The positive effect of using catch crops on SOC was observed in some periods at Foulum and Flakkebjerg, but any significant SOC enhancement appeared only at Flakkebjerg during 1997-2004 (Table 6). The inconsistences in SOC change between treatments with and without catch crops could not be explained by the constant higher C input in treatments with catch crops (Table 6). The advantage of enhanced C inputs from catch crops might have been compromised by the addition of rapidly decomposable materials in catch crops, leading to accelerated decomposition of more stable soil organic matter (Chen et al., 2014; Poeplau and Don, 2015). Moreover, changes in crop management and climate conditions could also influence decomposition (Kaspar et al., 2006; Steele et al., 2012). A large spatial variability in SOC also increases the difficulties in detecting small SOC changes after applying catch crops (Poeplau and Don, 2015).

Applying animal manure not only increased C input within animal manure itself, but also enhanced the quantity of crop residues, for the higher crop productivity under the enhanced nutrient supply (Millard and Angers, 2014). Even though the C input from different animal manures was very similar, only the cattle-sourced animal manures were observed to significantly enhance SOC (Table 6). This observation is consistent with the review by Maillard and Angers (2014), which found that animal manure of different animal species has different impacts, with cattle manure having a significant positive effect on SOC change, while pig and poultry manures do not. The significant, positive effects on SOC were also observed by Zavattaro et al. (2017) on a European scale, and the insignificant effect of pig slurry has been widely reported (Rochette et al., 2000; Plaza et al., 2004; Domingo-Olivé et al., 2016). The advantage of cattle-sourced animal manure has generally been ascribed to the presence of more stable organic matter (Velthof et al., 2000).

4.1.3 SOC change under organic farming and conventional farming

Organic farming generally produces lower yields than conventional farming (Connor, 2008; Leifeld et al., 2012; Seufert et al., 2012), and thus organic farming is assumed to transfer less C through photosynthesis to soil (Leifeld et al., 2013). In our study, however, even though the C input from aboveground residues was lower in organic farming, the C input from total plant residues was similar to that in conventional farming (Fig. 1b, Paper II). Moreover, there was a higher total C input in organic farming than in conventional farming systems (Table 5).

However, the higher C input in organic farming was not reflected in measured SOC change during the first four years of conventional farming (Table 6). This could indicate a slower microbial decomposition rate in the conventional soil during the the four years after conversion, considering that it had been converted from unfertilised organic farming in the previous eight years. Unfertilised soil has a much lower microbial biomass and enzyme activity than soil fertilised with animal manure (Plaza et al., 2004; Francioli et al., 2016), and the use of mineral fertilisers does not necessarily lead to a significant increase in either microbial biomass or enzyme activity over few years (Plaza et al., 2004). Another reason might be an overestimation of belowground C input in organic farming for the four years, since we estimated root C input based on data sampled in a limited number of years and species in both rotations.

From 2005-2012 at Foulum, SOC change reflected the differences in C input, indicating that the soil decomposition ability probably recovered after applying fertilisers for eight years. This was consistent with the 180-day incubation experiment started in 2015 at Foulum (Paper III), where the decomposition ability of soil from organic and conventional farming became very similar and not significantly different.

4.1.4 Recalcitrance of root vs shoots

Many studies report that C from belowground plant parts is better retained in soils than C from aboveground plant parts (Rasse et al., 2005; Kätterer et al., 2011; Berti et al., 2016). However, the results from regression analyses in both the arable rotation and incubation experiments showed that there were no differences between the recalcitrance of shoots and roots in the long term, but in the incubation experiment the shoots initially decomposed faster than the roots. A similar phenomenon was also reported for cover crops by Austin et al. (2017), where they observed in a ^{13}C labeled litter transfer experiment that aboveground materials decomposed faster than belowground materials in the first five months, whereas this difference disappeared after 17 months. Helfrich et al. (2008) also observed on maize that leaf litter decomposed faster in the short term, but no differences were found between leaf and root in the long term. This may be related to the chemical differences between above- and belowground materials (Rasse et al., 2005), resulting in different rates of organic matter decomposition in the initial phase, but not over the longer term (Gentile et al., 2011).

More N was mineralised from shoots than roots, and this could be ascribed to the higher N concentration and therefore lower C/N ratio in shoots, fitting the findings of Thomsen et al. (2016). Moreover, Jensen et al. (2005) found a close correlation between total pland N and net N mineralisation of plant materials.

4.1.5 Effects of soil management histories on soil decomposition ability

Management history showed no significant effect on C evolution from plant materials, which means that management history had little effect on the decomposing ability of microbes, even though there was significant lower total C, N and mineral N content in –CC+IF treatments (Table 2 and 3, Paper III).

Using the same plant material, more net N was mineralised when the material was added to soil with a history of catch crops (+CC) compared to soil without catch crops (-CC). This phenomenon may indicate that less of the mineralised N from catch crops was retained by microbes in the +CC soil (Jensen et al., 2005). This difference in characteristics of the microbial N mineralisation is also indicated by the higher total N and mineral N content for the +CC soils (Table 3, Paper III). These differences in N may originate from the improved N retention in the systems with systematic cultivation of catch crops for 10-18 years prior to sampling of the soil (Müller et al., 2006; Olesen et al., 2007; Thorup-Kristensen and Dresbøll, 2010). Constantin et al. (2011) in north France also observed an enhanced net N mineralisation in the field cultivated with catch crops for several years.

Results showed no difference in net N mineralisation of added plant material between soil amended with animal manure or inorganic fertiliser (Table 4, Paper III). Bosshard et al. (2009) also observed a similar lack of difference in N mineralisation, where labelled N in manure was applied to soils in organic and conventional rotations. This indicates that using catch crops can enhance soil total C and N level, especially in conventional farming (Table 2, Paper III), as well as mineral N content (Table 3, Paper III), and these enhanced soil properties could impact soil mineralisation properties.

4.2 Grassland system

4.2.1 Soil C and N change in grassland after conversion from arable land

Soil C

After 1998, the increase in soil C contents in grassland indicated three possible scenarios for the changes in C: 1) There was a larger C input in semi-natural grassland than in arable land, where organic matter decomposition could be faster, equal to or slower than in arable land; 2) equal or similar C input in semi-natural grassland compared to arable land, but slower organic matter decomposition than in arable land; or 3) less C input in semi-natural grassland than in arable land, and much slowr organic matter decomposition than in arable land.

Generally, converting arable land to grassland increases both the soil C content and labile pool of soil organic matter in the topsoil (Attard et al., 2016; Rosenzweig et al., 2016; Yu et al., 2017). Large amounts of labile C in soil can accelerate the decomposition of soil organic matter (Chen et al., 2014; Attard et al., 2016) such as from the incorporation of fresh or labile organic matter, while adding mineral N does not necessarily enhance organic matter decomposition (Booth et al., 2005; Chen et al., 2014). As reported by Attard et al. (2016), three years after converting arable land to non-legume-based grassland there was a 15-fold increase in root C in grassland compared to arable soil, and research has also shown faster organic matter decomposition in grassland (Acharya et al., 2012; Attard et al., 2016; Rosenzweig et al., 2016; Guo et al., 2017). Many studies also show a larger input from root materials in perennial grasses (e.g., Bolinder et al., 2012; Deru et al., 2016). Thus, the option 1) above is the more likely scenario, and the decrease in $\delta^{13}C$ indicated the increasing quantity of C from grasses (Table 8). The increased soil C in all treatments (Fig. 2) could also partly be ascribed to stopping tillage, since tillage not only disturbs the physical stability of soil (Powlson et al., 2014), but accelerates the decomposition of oxidizable labile C as well (Yu et al., 2017).

Following the larger C input after establishment and faster decomposition in grassland, there should an increasing quantity of labile C in grassland, which was partly reflected in the increase of soil C in the C-TOOL model (Fig. 4). The humification coefficient was assumed to be the same in grassland and arable land in C-TOOL, and lack of soil disturbance in the grassland phase main mean that the C from fresh materials and half-decomposed materials in grassland was underestimated in the modelling. As Attard et al. (2016) found, after conversion from arable land to grassland, C mineralisation did not differ much from arable land until 24 months after conversion. Thus, in our modelling, during grassland establishment the C inputs might be overestimated by not considering effects on decomposition rate.

Soil N

After conversion from arable land to grassland with no extra fertiliser input, the soil C and N contents in all treatments increased (Figs. 2a and 2b). Soil N followed the same pattern over time as C. Our aforementioned analysis of C change is therefore also suitable for N, where there should be a lower total N input from only atmospheric deposition, and a much lower N losses from leaching in grassland than in arable land. In arable land, applied N would leave soil through many ways, such as leaching, emission of N-containing gases and uptake by crops. Moreover, most of the aboveground crops were harvested in the arable phase. In constrast, grass was not harvested in semi-natural grassland, thus more N was cycled between plant and soil. In addition, many studies have reported that mineral N is scarce in natural grassland, giving a higher retention of N in the grassland system (Booth et al., 2005; Attard et al., 2016; Rosenzweig et al., 2016).

From the aspect of $\delta^{15}N$, in the low N losing grassland system, fractionation in N isotopes should also be supressed. Thus, sigatures of $\delta^{15}N$ in soil should become closer to the N from atmospheric decopistion, which was around 0.7 ± 2.3 ‰ in nitrate (Beyn et al., 2015), and even lower in ammonium (Freyer, 1978). As shown in Fig. 3b, $\delta^{15}N$ continually decreased in during semi-natural grassland phase.

4.2.2 Impact of cultivation history on soil C and N change in grassland

During the semi-natural grassland phase, the increments in soil C in three treatments were not significantly different. This may attributed to a generally low decomposition rate of original soil C (< 2.4 %) (Berti et al., 2016), a similar decomposition ability in added plant residues as aforementioned in the incubation experiment, and a similar C input based on similar nutrient supply, especially N, the primary limiting nutrient for the net primary production of plants (Jones et al., 2014). Since there were no legumes or artificial N inputs during the semi-natural grassland, new N inputs would primarily originate from atmospheric deposition, giving similar N inputs in grasslands of different pre-treatments.

Pre-treatments also had little impact on the increase in soil N. The semi-natural grassland was N limited and mineral N in soil became very scarce for both microbes and plants (Rosenzweig et al., 2016), thus N losses in semi-natural grassland system would be very low. Moreover, grasses were not harvested, and N in plant residues were therefore recycled through soil microbial degradation being affected by the N concentration in the plant residues. The observed soil N increase rate was 17±5 kg N ha^{-1} yr^{-1} (Table 7), which was similar to atmospheric N deposition of around 16 kg N ha^{-1} yr^{-1} reported by Ellermann et al. (2013). The phenomenon indicates that in a low leaking system like semi-natural grassland, the quantity of N in living plants migh be stable, while more N was returned and stored in soil within organic matter (Gregory et al., 2016; Rosenzweig et al., 2016). Therefore, after the establishment of semi-natural grassland, N from atmospheric was mainly stored in soil, and soil N increased similarly in soil with different pre-treamtents.

5 Perspectives

Our results show for Danish conditions the most practical and accurate estimates of root biomass are obtained by using fixed root amounts that depend on farming system and species (Table 7, Paper I). It would be useful to carry out similar analyses for other climatic and soil conditions, and for other types of farming systems. Based on our results, considering only farming systems for cereals would also provide an acceptable result without considering species differences. For catch crops and weeds separately, using fixed values under different farming systems could provide the best estimates. The observed differences in root biomass between years, especially in the upper soil layer, indicate that robust root biomass estimates should be based on measurements over several years. Our estimations of root biomass are generally comparable to other findings from other studies in Northern Euroupe (Braim et al., 1992; Kätterer et al., 1993; Van Noordwijk et al., 1994; Pietola and Alakukku, 2005; Głąb et al., 2014), indicating the general availability in areas under similar climate. The approach also indicated different estimated belowground C inputs in various arable management systems, thus more research is needed to quantify belowground biomass in many different management systems for better estimation of the C balance in various systems.

Using green manure, catch crops and animal manure each enhanced C input by 0.6-1.0 Mg C ha^{-1} yr^{-1} at crop rotation level. However, only the effect of green manure was consistently reflected in the observed SOC change. This indicates a need for studies on C inputs from various sources and how the C inputs are degraded and contribute to stabilised SOC. Better estimation of C inputs from roots and root exudates is also highly needed to assess the effect of cropping systems and crop management on SOC (Keel et al., 2017). Moreover, the C inputs from fresh organic materials, as an energy supplier for microbes, may accelerate SOC decomposition (Chen et al., 2014). Thus, there should also be considerations on the selections of C input e.g. perennial grasses, which provide less energy and keep the SOC decomposition at a lower level.

The inclusion of grass crops in the crop rotation has been widely reported to increase soil C (Leifeld et al., 2012; Taghizadeh-Toosi and Olesen, 2016). However, grassland management also has considerable effects on SOC (Conant et al., 2017), and this should be considered when evaluating the effects of organic farming systems on total greenhouse gas emissions. This is illustrated by the effect in our study of removing grass-clover cuttings as compared to mulching these in the field. This removal of grass-clover in the experiment was meant to simulate a situation where the grass-clover was used for biogas and the digested slurry was used to fertilise the other crops in the rotation. This approach enhanced yields of the arable crops in the rotation by about 30% (Shah et al., 2017), which also enhanced C inputs from crop residues (Fig. 1b, Paper II). However, this could not make up for the C not returned in mulched grass-clover. It is therefore necessary to find other agronomical measures for both high yields and high soil C inputs, e.g. applying mineral fertilisers in farming systems with grasslands.

Our study showed higher estimated C inputs in organic compared with similar conventional cropping systems (Table 5), whereas measured SOC changes showed opposite trends, although this trend was reversed over time at Foulum (Table 6). This contrasts with other studies that showed consistently higher SOC accumulation in organic compared with conventional systems (Gattinger et al., 2012). In reality, there may be considerable variation between different organic and conventional stratagies in their ability to enhance SOC, and measures such as using green manure, catch crops and residue management need further research under specific circumstances in better enhancing SOC.

C inputs from root inputs may enhance soil C more than shoot inputs in the short term. However, we could not confirm that root C inputs would generally enhance soil C more than shoot C, which aligns with assumptions commonly used in soil C models (e.g. Taghizadeh-Toosi et al. 2014a). Additionally, plant material added to soil of different management histories showed similar C decomposition rates (Table 3, Paper III). Generally, with incubation over 180 days showed no difference in C retained in soil from added root or shoot plant material. Therefore, higher quantity of C input is the first consideration in enhancing soil C stock for the long-term, rather than prehistory or the type of plant C input (above- or belowground).

The use of catch crops, in particular legume-based catch crops in the organic farming systems, increased the rate of soil N mineralisation (Petersen et al., 2013), which was reflected in higher measured soil mineral N (Table 1, Paper III). This higher N mineralisation helps to increase aboveground biomass and crop yields (Shah et al., 2017), thus leading to further C input and therefore higher soil C stocks. The contribution of catch crops to soil organic C is therefore not only through the C input from catch crops themselves.

Converting arable land to grassland (fertilised or unfertilised) is an effective soil C sequestration method as found in many studies, with annual accumulation ranging from 0.3-1.9 Mg C ha^{-1} yr^{-1} (Christensen et al., 2009; Müller-Stöver et al., 2012; Rosenzweig et al., 2016). Compared to the general decrease of C in soil, using semi-natural grassland increased C contents by 0.39 Mg C ha^{-1} yr^{-1} in the topsoil, indicating the extra benefit of C sequestration from the atmosphere, and that these benefits were little influenced by different cultivation histories. Moreover, the accelerated mineralisation in grassland compared with arable land, as mentioned in many papers (Booth et al., 2005; Acharya et al., 2012; Attard et al., 2016; Rosenzweig et al., 2016; Guo et al., 2017), indicated the underestimation of humification coefficients in C-TOOL model for grassland. Such indication could be useful in order to improve modelling of both arable land and grassland.

6 Conclusions

The statistical analysis of collected root biomass data from field experiments in Denmark indicated that in cereal crops, catch crops and weeds, farming systems and species were the key factors for predicting root biomass. Fixed root biomass estimations based on farming system provided the most robust estimations.

Using green manure gave a higher C input of about 1.0 Mg C ha^{-1} yr^{-1}, and showed a consistently greater retention of SOC. However, this ability could be compromised if the aboveground biomass of green manure was removed. Catch crops contributed an approximately 1.0 Mg C ha^{-1} yr^{-1} greater C input, whereas no general, positive effect on SOC retention could be observed. Neither was there a general advantage for SOC retention of applying animal manure, even though animal manure across all locations added an extra 0.7 Mg C ha^{-1} yr^{-1} to soil, where over 0.4 Mg C ha^{-1} yr^{-1} came from C in crops.

Compared with conventional farming, organic farming systems had larger total C inputs, in particular from more belowground plant material and added animal manure. However, the higher C input was not necessarily reflected in a measureable change in SOC.

The belowground plant part had the potential to contribute more of its C to SOC in the short term compared to the aboveground part, but this difference may become insignificant in the longer term. However, there was more N mineralised and released from shoots than roots due to a higher N concentration in shoots.

After more than 10 years of continuous management histories, previous soil management (with or without catch crops and with animal manure or mineral fertilisers) showed no significant effect on decomposition of C in added plant material, while soil with a previous management history of catch crops had a higher net N mineralisation of the N in added plant material.

At the experiment at Askov, soil C and N contents increased in semi-natural grassland at rates of 0.29 Mg C ha^{-1} yr^{-1} and 17 kg N ha yr^{-1}. Simultaneously, $\delta^{13}C$ and $\delta^{15}N$ signatures dropped individually at rates of 0.063 ‰ yr^{-1} and 0.074 ‰ yr^{-1}. However, the pre-treatment during the arable stage did not effect the change of soil C and N contents or isotopic signatures during the grassland stage.

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Paper I

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Research Paper

Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass

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ABSTRACT

Reliable information on belowground plant biomass is essential to estimate belowground carbon inputs to soils. Estimations of belowground plant biomass are often based on a fixed allometric relationship of plant biomass between aboveground and belowground parts. However, environmental and management factors may affect this allometric relationship making such estimates uncertain and biased. Therefore, we aimed to explore how root biomass for typical cereal crops, catch crops and weeds could most reliably be estimated. Published and unpublished data on aboveground and root biomass (corrected to 0–25 cm depth) of cereal crops (wheat and barley), catch crops and weeds were collected from studies in Denmark. Leave one out cross validation was used to determine the model that could best estimate root biomass.

Root biomass varied with year, farming system (organic versus conventional) and cereal species. Shoot and root biomass of catch crops were higher than for weeds (sampled in late autumn), and farming system significantly affected root biomass of catch crops and weeds. The use of fixed root biomass based on the most influential factors (farming system and species) provided the lowest error of prediction for estimation of root biomass, compared with the use of fixed allometric relations, such as root/shoot ratio. For cereal crops, the average root dry matter in organic farming systems was 218 g m^{-2} (243 and 193 g m^{-2} for wheat and barley, respectively), but in conventional systems only 139 g m^{-2} (142 and 129 g m^{-2} for wheat and barley, respectively). For catch crops and weeds, the root dry matter in organic farming systems were around 127 and 35 g m^{-2}, and in conventional farming systems 75 and 28 g m^{-2}, respectively.

In conclusion, the present analysis indicates that root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass, and it may be better estimated using fixed values based on species and farming systems than using fixed allometric ratios.

1. Introduction

Soil fertility in agricultural systems is sustained through inputs of organic matter from plant residues and from applied manure and compost (Lal, 2004a,b). These inputs contribute to carbon (C) storage and sequestration in soils, which in some cases may help to mitigate other greenhouse gas emissions (Powlson et al., 2011). The plant inputs of C from both aboveground and belowground components are generally calculated from their plant biomass by multiplying with specific transfer (humification) coefficients (Chirinda et al., 2012; Kätterer et al., 2011). However, unlike aboveground plant biomass, root biomass is difficult to sample and quantify. The C originating from roots can represent an important source for soil C storage (Warembourg and Paul, 1977), not least because they may contribute more to stable soil organic C (SOC) pools than aboveground inputs (Kätterer et al., 2011). Such considerations suffer from the fact that the amount of belowground C inputs is mostly not well quantified under field conditions (Smucker, 1984; Taylor, 1986). The difficulties in measuring belowground C inputs means that other approaches have to be taken to estimate this component. Therefore, simple estimation methods have been proposed for estimating belowground C inputs, and these are used for accounting purposes and in many cases also for soil C modelling (Keel et al., 2017).

Allometric estimation of root C inputs, where a certain (often constant) proportion of plant dry biomass is allocated to the root, is a commonly used method, for instance in national inventories of soil C changes (Johnson et al., 2006). Estimating root biomass using fixed allometric ratios is based on the assumption that for specific species and environmental conditions, growth of roots and shoots are closely associated

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(Pearsall, 1927; Poorter and Nagel, 2000). This assumes that the biomass allocated to roots is proportional to shoot biomass with a ratio determined by plant species and environmental conditions. As a consequence, the proportion is often a key parameter to estimate root biomass of crops under similar conditions. However, the ratio between the root and aboveground biomass varies between species and depends on environmental conditions (Bolinder et al., 1997, 2007; Campbell et al., 2000).

Many studies have shown that the proportion of the net primary productivity that is allocated to the belowground part is sensitive to the environmental conditions, e.g. nutrient and water availability and tillage (Hodge et al., 2000; Muñoz-Romero et al., 2009). Increasing N application will increase the growth of shoots, while N fertilisation has little effect on root biomass (Jenkinson, 1981; Anderson, 1988; Huggins and Fuchs, 1997). Thus shoots and roots respond differently to particular environmental conditions. Even though the allometric ratio has been shown to vary considerably (Johnson et al., 2006; Gyldenkærne et al., 2007), it is widely used to estimate root biomass, e.g. in models of soil carbon inputs (Kätterer et al., 2011; Berti et al., 2016). Although there is some evidence showing that root biomass seem to be constant for a certain species in a particular environment rather than varying if estimated from shoot biomass using a fixed allometric relationship (Chirinda et al., 2012), this assumption has not been thoroughly tested.

Given the large uncertainties in current methods for estimating root C inputs, our objective was to compare methods for root biomass estimation, in particular the fixed allometric functions versus fixed root biomass. In this analysis we also explore which environmental and management factors affected shoot and root biomass of cereals, catch crops and weeds.

2. Methodology

Published and unpublished shoot and root biomass data from several field experiments in Denmark were collected. Mean values of each treatment were used to obtain statistically equal weight between treatments, and the data covered both cereal crops (Table 1) and catch crops and weeds (Table 2).

2.1. Cereals

2.1.1. Description of experiments

Data for cereal crops (winter and spring wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.)) was collected from studies conducted at Foulum (56°30′N, 09°35′E) in western Denmark. Organic and conventional farming systems at Foulum showed no overall differences in topsoil (0–25 cm) properties, which was loamy sand soil (Typic Hapludult) with clay content of 88 g kg^{-1}. The soil pH was 6.5. Organic matter content was 38 g kg^{-1}. Soil bulk density was 1.42 g cm^{-3}. Average annual temperature and precipitation during 1961–1990 were 7.3 °C and 704 mm. More information on soil properties was provided by Olesen et al. (2000).

Data from 2008 and 2010 were sampled in a long-term crop rotation experiment initiated in 1997 (Olesen et al., 2000). Briefly, the experiment included two rotation systems, one inorganic fertiliser-based conventional system and one organically managed system in two replicates. All treatments were ploughed (Table 1). More information on field management is given in Chirinda et al. (2012).

Data from 2013 and 2014 were sampled in a field experiment established in 2002 under conventional management with four replicates. Generally, there were two factors: nitrogen fertiliser rates and tillage (ploughing and no tillage) (Table 1). In 2013, nitrogen rates were 50 and 250 kg N ha^{-1}, while in 2014 they were 65 and 265 kg N ha^{-1} for the same sub-plots. More details on the experiment are given in Munkholm et al. (2008) and Hansen et al. (2011).

The mean climatic conditions during the spring period (March to May) are shown for these experimental years in Table 3. The potential evapotranspiration was calculated using a modified Makkink method (Hansen, 1984) using temperature and global radiation as determining variables.

Table 1
Shoot dry matter at maturity and root dry matter at anthesis in field studies with cereals at Foulum, Denmark.

Species	Shoot (Maturity) (g m^{-2})	Root (Anthesis) (g m^{-2})	Sampling depth (cm)	Root corrected to 0–25 cm g m^{-2}	Root/(Shoot + Root) 0–25 cm	Year	Seeding time	Farming system	N applied (kg ha^{-1})	Tillage	Reference
Wheat	1907	204	30	194	0.09	2008	Autumn	Conventional	165	Ploughed	Chirinda et al. (2012)
	838	213		203	0.19			Organic	0		
	1271	249		236	0.16				102		
	1145	291		277	0.19				108		
	1482	251		239	0.14				108		
	1124	156	30	148	0.12	2010	Spring	Conventional	110		
	1350	187		177	0.12				110		
	1093	322		306	0.22			Organic	102		
	1171	211		201	0.15				102		
	1175	116	20	124	0.10	2013	Autumn	Conventional	50	Ploughed	Sharif et al. (Submitted)
	1571	86		92	0.06				250		
	1226	123		131	0.10				50	No-tillage	
	1613	99		106	0.06				250		
	1283	154	20	165	0.11	2014		Conventional	65	Ploughed	
	1673	148		159	0.09				265		
	1266	128		137	0.10				65	No-tillage	
	1614	120		129	0.07				265		
Barley	1135	153	30	146	0.11	2008	Spring	Conventional	130	Ploughed	Chirinda et al. (2012)
	965	238		226	0.19			Organic	0		
	772	200		190	0.20				57		
	1043	236		224	0.18				57		
	1271	240		228	0.15				57		
	1267	140	30	133	0.09	2010		Conventional	120		
	1251	113		108	0.08				120		
	982	162		154	0.14			Organic	62		
	987	142		135	0.12				62		

Table 2
Shoot and root dry matter measured in fields with catch crops and weeds in Denmark.

Species	Shoot ($g\ m^{-2}$)	Root ($g\ m^{-2}$)	Sampling depth (cm)	Root corrected to 0–25 cm $g\ m^{-2}$	Root/(Shoot + Root) 0–25 cm	Location	Sampling procedure	Farming system	Legume based or not[a]	Sowing time[b]	Reference
Fodder radish	170	130	18	147	0.46	Foulum	Excavation	Organic	NL	Autumn	Li et al. (2015)
Perennial ryegrass	130	130		147	0.53				LB	Spring	
Red clover	190	140		158	0.45					Spring	
Ryegrass/clover mix	190	120		135	0.42					Spring	
Winter vetch	170	120		135	0.44					Autumn	
Ryegrass/clover mix	207	153	30	143	0.41	Foulum	Soil cores	Organic	LB	Spring	Chirinda et al. (2012)
	271	144		135	0.33					Spring	
Fodder radish	470	90	20	98	0.17	Aarslev	Excavation	Organic	NL	Autumn	Thorup-Kristensen (2001)
Winter rape	400	140		152	0.28					Autumn	
Phacelia	420	50		54	0.11					Autumn	
Rye	210	100		108	0.34					Autumn	
Oats	310	70		76	0.20					Autumn	
Italian ryegrass	350	190		206	0.37					Autumn	
Malva sylvestris	360	200		217	0.38					Autumn	
Agrostemma githago	530	100		108	0.17					Autumn	
Rye/vetch mix.	330	140		152	0.32				LB	Autumn	
Winter vetch	370	60		65	0.15					Autumn	
Fodder radish	200	72	20	78	0.30	Foulum	Excavation	Conventional	NL	Spring	Mutegi et al. (2011)
Fodder radish	219	108		117	0.35					Spring	
Fodder radish	267	46	20	50	0.16	Flakkebjerg	Soil cores	Conventional	NL	Autumn	Unpublished
Radish/Rye mix	629	81	20	87	0.12	Foulum	Excavation	Conventional	NL	Spring	Unpublished
	184	41		44	0.19					Spring	
Radish/Ry/Vetch mix	565	96		104	0.16			Organic	LB	Spring	
Chicory/clover mix	85	63		69	0.45					Spring	
Weeds	85	78	30	73	0.46	Foulum	Soil cores	Conventional	–	–	Chirinda et al. (2012)
	53	78		73	0.58			Organic			
Weeds	262	4	20	4	0.02	Foulum	Excavation	Conventional	-	-	Unpublished
	47	8		8	0.15						
	208	8		9	0.04			Organic			
	45	22		24	0.34						

[a] NL, non-legume; LB, legume-based.
[b] Spring, catch crops were undersown in preceding cereal crops; Autumn, catch crops were sown after harvest of the cereals.

Table 3
Climatic conditions during spring (March to May) at Foulum during the experimental years.

Year	Mean temperature (°C)	Mean daily global radiation ($MJ\ m^{-2}$)	Precipitation (mm)	Potential evapotranspiration (mm)
2008	7.4	15.0	134	209
2010	5.4	12.9	106	169
2013	5.3	14.6	85	188
2014	8.5	13.5	157	191

2.1.2. Measurements

Shoot biomass was sampled at maturity, and root biomass was sampled at anthesis as this is the growth stage expected to have maximum root biomass. Plant samples of aboveground biomass were taken by cutting plants at 1–2 cm height within two 0.5 m^2 frames. Samples were oven dried at 60 °C for 48 h for dry matter (DM). Three soil cores (5 cm diameter) were collected within the rows and three between the rows for root biomass. Root sampling reached 30 cm depth in 2008 and 2010, and 20 cm in 2013 and 2014. Samples to 60 cm depth were also taken in 2008, 2013 and 2014. The root samples were washed out using tap water and collected on a sieve with a mesh size of 0.425 mm. Samples were oven dried at 70 °C for 48 h and weighed for dry matter. A part of the root sample was heated at 650 °C for five hours to determine the ash content, and final root dry matter was expressed as ash-free dry matter (Chirinda et al., 2012).

2.2. Catch crops and weeds

2.2.1. Description of experiments

Data on catch crops (fodder radish (*Raphanus sativus* L.), perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), winter vetch (*Vicia villosa* Roth.), winter rape (*Brassica napus* L.), phacelia (*Phacelia tanacetifolia* Benth.), rye (*Secale cereale* L.), oats (*Avena sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), *Malva sylvestris* L., *Agrostemma githago* L. and chicory (*Cichorium intybus* L.)) were collected from Mutegi et al. (2011) in four replicates, Chirinda et al. (2012) in two replicates, Li et al. (2015) in three replicates sampled at Foulum (56°30′N, 09°35′E), from Thorup-Kristensen (2001) in three replicates at Aarslev (55°18′N, 10°27′E), and from Wahlström et al. (2015) in four replicates at Flakkebjerg (55°19′N, 11°23′E) (Table 2). Topsoil (0–25 cm depth) at Foulum is described above for cereals crops. Topsoil of the same depth at Aarslev and Flakkebjerg were both classified as sandy loam (Typic Agrudalf) with clay content of 147 g kg^{-1} at both sites, and pH 7.0 and 7.4, respectively (Thorup-Kristensen, 2001; Olesen et al., 2000). The average annual temperature and precipitation were 8.1 °C and 719 mm (during 1986–1998) at Aarslev (Mueller and Thorup-Kristensen, 2001), and 7.8 °C and 626 mm (during 1961–1990) at Flakkebjerg (Olesen et al., 2000).

Published data from Foulum (Chirinda et al., 2012; Li et al., 2015) was sampled from cropping systems under organic farming, except for weeds sampled in the inorganic fertiliser-based rotation system in Chirinda et al. (2012). The data from Li et al. (2015) included two legume-based catch crops. Data from Aarslev was from a cropping system with vegetables under organic farming, where catch crops were sown after the harvest of green pea crops. Two of the treatments included legume-based catch crops with winter vetch (Thorup-Kristensen, 2001). The data from Flakkebjerg were from fodder radish sown after the harvest of spring barley in a conventionally managed cropping system (Wahlström et al., 2015).

2.2.2. Measurements

At Foulum, Mutegi et al. (2011) sampled fodder radish in December by clipping the aboveground biomass at the soil surface from four subplots of 0.64 m^2, and by extracting root from three soil cores in each replicate to 100 cm depth. Samples were then sub-divided at 20 cm, 35 cm and 60 cm depths. Chirinda et al. (2012) used the method for cereal crops also to measure catch crops in early November. Li et al. (2015) sampled catch crop roots in small frames (35 × 24 cm) down to 18 cm. The area covered two rows of catch crops. The root washing procedure was the same as in Chirinda et al. (2012). At Aarslev, aboveground parts of catch crops were sampled in 1 m^2 just below ground level, and roots were washed out from two excavated soil blocks of 30 × 12 cm^2 area and 20 cm depth in November (Thorup-Kristensen, 2001). Only visibly live roots were retained. At Flakkebjerg, aboveground parts of catch crops were sampled at soil surface in two 0.25 m^2 areas in November, and roots were sampled from three soil cores (8.6 cm diameter) vertically down to 100 cm depth, and subdivided at 20 cm, 35 cm, 55 cm and 80 cm depths (Wahlström et al., 2015).

To supplement these data, additional data were collected from catch crops and weeds sampled in December 2014 in the aforementioned long-term organic crop rotation experiment at Foulum (Olesen et al., 2000) in two replicates. Three types of catch crops following potato and spring wheat were sampled for shoot and root biomass. These catch crops were mixtures of species, i.e. fodder radish + rye, fodder radish + rye + vetch, chicory + perennial ryegrass + red clover + white clover. Also sampling was made in plots without catch crops, but with weeds. Shoots were separated on the basis of species, while roots were analysed as a pooled sample. A square of 0.5 m^2 was used for sampling of aboveground material in each plot. Inside the 0.5 m^2 square, an area of 35 × 24 cm^2 was chosen from within and from midway between crop rows. Aboveground plants inside the 35 × 24 cm^2 area were cut with scissors at the soil surface and collected in a plastic bag, whilst the remaining sample inside the 0.5 m^2 was collected in a second bag. Each sample was separated according to species groups and dry matter was determined after oven drying at 60 °C for 42 h. Belowground biomass was determined for the 35 × 24 cm^2 area to a depth of 20 cm in each plot. The soil samples were stored at 2 °C before root washing.

The roots were first separated from the soil by passing through a 1-cm sieve. Large visible roots and those retained on the 1 cm sieve were collected, termed ‘large roots’. The bulk soil passing the 1 cm sieve was mixed and subdivided into a subsample of 350–450 g, which was washed on a 0.425 mm sieve. The roots collected on this sieve are termed ‘small roots’ (Rasmussen et al., 2010). Roots were further washed with tap water to remove minerals and collected on a set of sieves with mesh sizes of 2 mm, 1 mm and 0.425 mm. Subsequently, the collected roots and debris were placed in a tray, where white living roots were separated from dead organic matter (including decayed roots) based on colour and physical appearance (Muñoz-Romero et al., 2009). Living roots were oven-dried at 60 °C for 42 h and weighed. A part of each root sample was heated at 650 °C for five hours to determine the ash content, and final root dry matter was expressed as ash-free dry matter (Chirinda et al., 2012).

2.3. Root biomass depth correction

Different farming systems and N managements showed little impact on vertical root biomass distribution of either cereal crops or catch crops and weeds (See Supplementary materials Table S1 in the online version at DOI: 10.1016/j.agee.2017.09.024), and similar results were also reported in Hirte et al. (2017). Since roots were sampled to different depths in the various studies, we applied two different functions for the depth correction, one for cereals (Eq. (1)) and another for catch crops and weeds (Eq. (2)). This choice was based on previous studies and on available data. This was as far as possible validated against root biomass data from different depths reported in Supplementary material. Root dry matter measurements of cereal crops were converted to 25 cm depth according to the Michaelis-Menten function of root distribution with depth (z; cm) as used in Kätterer et al. (2011) for root depth distribution of small-grain cereals in southern Sweden.

$$\mathrm{Rm}(z) = [z\,(z_{50} + z_r)]/[z_r\,(z_{50} + z)] \quad (1)$$

Rm(z) is the fraction of total root mass to the soil depth of z (cm), z_r is maximum root depth (z_r was set at 150 cm), z_{50} is the depth of 50% of the root mass (z_{50} was for cereals in Sweden set at 10 cm). This means that 76, 80 and 91% of the roots are allocated to 25 cm, 30 cm and 60 cm soil depth, respectively. In this function, 88% of root biomass in 0–60 cm depth was estimated for 0–30 depth, which was close to the root vertical distribution of cereals in years 2008 and 2014 (Table S1).

Roots of fodder radish sampled in Flakkebjerg were classified into 5 depths: 0–20, 20–35, 35–55, 55–80 and 80–100 cm (Wahlström et al., 2015). Within 100 cm depth, recoverable root dry matter of catch crops in different depths was well described as (See Fig. S1 in the online version at DOI: 10.1016/j.agee.2017.09.024):

$$\mathrm{Rm}(z) = 0.1926\ z^{0.3641} \quad (2)$$

According to Eq. (2), in soil depths of 25, 30 and 60 cm, root dry matter accounted for 62, 66 and 86%, respectively, of total root biomass in the upper 100 cm soil. This meant that 78% of the root present in 0–60 cm depth was recovered in 0–30 cm layer. This corresponded well to the root distribution observed for catch crops (with mainly ryegrass) and weeds, where the proportion of recoverable root biomass from 0 to 30 cm depth compared to biomass in 0–60 cm was between 68 and 77% (Chirinda et al., 2012). Thus, the equation was assumed suitable and was used to convert root dry matter of catch crops and weeds from the measured depths to 0–25 cm depth.

2.4. Data analysis

The MIXED procedure of SAS (SAS Institute, 1996) was used to test which factors influence crop shoot, root and the allometric ratios (root/shoot, shoot/root, shoot/(shoot + root) and root/(shoot + root) ratio): year, species (wheat or barley), seeding time (spring or autumn), tillage (ploughing or no tillage), farming system (organic or conventional management) and nitrogen fertilisation rate, where shoot biomass, root biomass and nitrogen fertilisation rate were used as continuous variables and other variables were categorical. We thus assumed that allometric ratios would depend on plant type and management. These allometric functions essentially assume linear relations of root biomass to either shoot or total biomass. For catch crops and weeds the following factors were considered: location, catch crops or weeds, legume based or non-legume based catch crops, undersowing catch crops or sowing these after

Table 4
Factors affecting shoot, root biomass and their allometric ratios, and comparison of methods for estimating root biomass using cross-validation (LOOCV) for cereals, N = 26.

Target variables	P values for influential factors[a]						LOOCV for root estimation methods based on most influential factors in	
	Year	Species	Seeding time[b]	Farming system	Nitrogen	Tillage	MBE_P ($g\ m^{-2}$)	$RMSE_P$ ($g\ m^{-2}$)
Shoot			0.0205		< 0.0001			
Shoot of cereal crops		–	0.0205		< 0.0001			
							Root estimation by fixed root amount	
Root	0.0154	0.0022		0.0013			0.0	33.3
Root ignoring year	–	0.0347		< 0.0001			−0.3	37.6
Root of cereal crops		–		< 0.0001			0.0	39.8
Root (Species)	–	0.7850	–	–	–	–	0.0	57.8
Root (Species, seeding time)	–	0.2831	0.2248	–	–	–	0.0	59.7
							Root estimation by root/shoot ratio	
Root/shoot ratio				< 0.0001			3.4	42.7
Root/shoot ratio of cereal crops		–		< 0.0001			3.4	42.7
Root/shoot ratio (Species)	–	0.3504	–	–	–	–	10.2	78.1
Root/shoot ratio (Species, seeding time)	–	0.7196	0.1702	–	–	–	9.2	77.0
							Root estimation by shoot/root ratio	
Shoot/root ratio	0.0008	0.0023	0.0222	0.0018	< 0.0001		2.0	42.6
Shoot/root ratio ignoring year	–			0.0002	0.0005		−3.5	43.4
Shoot/root ratio of cereal crops	0.0090	–		0.0064	0.0001		−1.0	45.5
Shoot/root ratio (Species)	–	0.2242	–	–	–	–	−17.6	75.4
Shoot/root ratio (Species, seeding time)	–	0.7495	0.1099	–	–	–	−15.5	73.4
							Root estimation by shoot/all or root/all ratio	
Shoot/all or root/all ratio	0.0366	0.0047	0.0166	0.0001	0.0155		1.7	38.2
Shoot/all or root/all ratio ignoring year	–			< 0.0001	0.0309		0.5	38.5
Shoot/all or root/all ratio of cereal crops		–		< 0.0001	0.0309		0.5	38.5
Shoot/all or root/all ratio (Species)	–	0.3239	–	–	–	–	6.3	77.1
Shoot/all or root/all ratio (Species, seeding time)	–	0.7325	0.1616	–	–	–	5.7	75.9

[a] Factors with '–' were not included in the statistical analysis for influential factors. Blank cells were items included in the statistical analysis, but not statistically significant ($p > 0.05$). Factors shown in p values were used for leave one out cross validation (LOOCV). Factors in brackets mean the only factors considered for LOOCV.
[b] Seeded in spring or autumn.

harvest of the main crop, and farming system. A manual procedure with backward elimination was used to remove variables that did not contribute significantly based on the Akaike Information Criterion (AIC). The best model was thus selected according to the lowest AIC and significant ($P < 0.05$) effect of independent variables.

Different approaches (allometric functions and various determining factors) for estimating root biomass were tested by leave one out cross validation (LOOCV) based on mean bias error (MBE) and root mean squared error (RMSE). The models chosen for testing were based on the selected models using the stepwise procedure described above. Specific equations are shown as below:

$$MBE_P = \frac{\sum_{i=1}^{n} (P_i - O_i)}{n} \quad (3)$$

$$RMSE_P = \sqrt{\frac{\sum_{i=1}^{n} (P_i - O_i)^2}{n}} \quad (4)$$

where MBE_P and $RMSE_P$ means MBE and RMSE of prediction for the selected models for LOOCV with total population of samples as n, P_i is the predicted root dry biomass of sample i through the selected model trained by all other samples, and O_i is the observed root dry biomass of sample i.

3. Results

3.1. Factors affecting shoot and root biomass

Shoot biomass of cereals was strongly influenced by the quantity of nitrogen applied in mineral fertiliser or manure. The shoot biomass varied between spring and winter cereals, while root biomass varied between years and depended on farming system (organic or conventional) and cereal crop species (Table 4). Thus shoot and root dry biomass was not closely associated, but influenced by different factors. In addition, the different allometric ratios responded differently to determining factors. Root/shoot ratio was sensitive to the type of farming system, while shoot/root ratio, shoot/all and root/all were influenced by several factors, i.e. year, species, sowing time, farming system and nitrogen rate. Therefore, the most reliable estimates of root biomass depend on farming system and species with higher root biomass in organic compared with conventional systems (Table 5).

Table 5
Mean root dry biomass (to 25 cm depth) measured in cereals, catch crops and weeds at Foulum, Denmark (data from Tables 1 and 2).

	Farming system	Species	Root biomass[a] ($g\ m^{-2}$)	N
Cereals	Organic	Wheat	243 ± 41	6
		Barley	193 ± 40	6
		Cereals	218 ± 47	12
	Conventional	Wheat	142 ± 30	11
		Barley	129 ± 19	3
		Cereals	139 ± 28	14
Catch crops and weeds	Organic	Catch crops	127 ± 44	19
		Weeds	35 ± 34	3
	Conventional	Catch crops	75 ± 29	5
		Weeds	28 ± 38	3

[a] Mean ± S.D.

When pooling data over all years and cereal species, the root biomass only responded significantly to farming system, whereas shoot/root ratio as well as shoot/all and root/all ratios depended mostly on farming system and nitrogen rate.

There were significant differences between catch crops and weeds for both shoot and root biomass (Table 6). Root biomass was affected by type of farming system. Root/shoot ratio depended on location and farming system, while shoot/root ratio varied between catch crops and weeds. Shoot/all or root/all ratios were not significantly affected by any factors.

Table 6
Factors affecting shoot, root biomass and their allometric ratios, and comparison of methods for estimating root biomass of catch crops and weeds using cross-validation (LOOCV).

Target variables	P Value for influential factors[a]					Test for root estimation methods based on most influential factors in LOOCV		
	Location	Farming system	CC or not[b]	LB or not[c]	Undersown or not[d]	MBE_P ($g\ m^{-2}$)	$RMSE_P$ ($g\ m^{-2}$)	N
Shoot			0.0062					
						Root estimation by fixed root amount		
Root		0.0347	0.0009			0.2	43.0	30
Root (CC or not, Farming system)	–	0.0347	0.0009	–	–	0.2	43.0	30
Root (CC or not, LB or not)	–	–	–	0.6430	–	0.0	46.3	30
						Root estimation by root/shoot ratio		
Root/shoot ratio	0.0294	0.0138				20.8	87.8	29
Root/shoot ratio (CC or not, Farming system)	–	0.0885	0.5707	–	–	30.3	90.5	30
Root/shoot ratio (CC or not, LB or not)	–	–	–	0.2133	–	29.3	92.2	30
						Root estimation by shoot/root ratio		
Shoot/root ratio			0.0116			−18.5	67.2	30
Shoot/root ratio (CC or not, Farming system)	–	0.2819	0.0279	–	–	5.7	80.9	30
Shoot/root ratio (CC or not, LB or not)	–	–	–	0.2196	–	−15.9	71.8	30
						Root estimation by shoot/all or root/all ratio		
Shoot/all; root/all ratio						11.7	76.6	30
Shoot/all; root/all ratio (CC or not, Farming system)	–	0.1060	0.9298	–	–	17.2	80.9	30
Shoot/all or root/all ratio (CC or not, LB or not)	–	–	–	0.1796	–	15.5	83.5	30

[a] Factors with '–' were not included in statistical analysis for influential factors. Blank cells were items included in the statistical analysis, but not statistically significant ($p > 0.05$). Factors shown in p values were used for leave one out cross validation (LOOCV). Factors in brackets mean the only factors considered for LOOCV.
[b] CC or not means catch crops or weeds.
[c] LB or not means legume based or non-legume based catch crops.
[d] Undersown or not means undersowing or not undersowing catch crops.

3.2. Root estimation methods

Different methods for estimating root biomass were tested by cross validation and evaluated in terms of MBE_P and $RMSE_P$ using cross validation (Table 4). The most reliable predictions of soil root biomass were obtained for cereals using fixed root amount with mean biomass values depending on year, farming system and species giving an $RMSE_P$ of only 33 $g\ m^{-2}$ (Table 4). The second best method was using fixed root biomass depending on farming system and cereal species with $RMSE_P$ of 38 $g\ m^{-2}$. Fixed root estimation which only considered farming system provided the simplest estimation, but with a $RMSE_P$ of 40 $g\ m^{-2}$. Grouping data according to species, or species and sowing time (autumn or spring) reduced the performance of root biomass prediction (i.e. higher $RMSE_P$ of the cross-validation). Estimation of root biomass based on shoot biomass with allometric relations according to root/shoot, shoot/root or even shoot/all (root/all) ratio showed either poorer prediction performance and/or was more complex than using fixed root biomass.

Table 7
Root biomass estimated by least square means (0–25 cm). Same data as used in Table 5.

	Class	Variables	Root dry biomass[a]($g\ m^{-2}$)
Cereal crops	Farming systems	Organic	189 ± 13
		Conventional	131 ± 9
	Species	Wheat	184 ± 8
		Barley	135 ± 12
	Year	2008	199 ± 10
		2010	170 ± 10
		2013	118 ± 18
		2014	152 ± 18
Catch crops and weeds	Farming systems	Organic	88 ± 11
		Conventional	49 ± 15
	Species	Catch crops	105 ± 10
		Weeds	32 ± 17

[a] Mean ± S.D.

The most reliable estimates of root biomass in catch crops and weeds were obtained by using fixed root biomass for catch crops and weeds separately for different farming systems (Table 6). Adding factors such as catch crop characteristics (e.g. legume based) did not improve predictions. Similar to the cereal crops, using allometric relationships reduced the prediction accuracy for root biomass in catch crops and weeds.

3.3. Fixed root biomass estimation

According to the results above, we suggest using fixed root biomass classified by farming systems and species for cereals, and by farming systems for catch crops and weeds (Table 5). Table 7 shows the estimated root biomass by least square means taking into account the most influential factors for cereals (farming systems, species and year), and for catch crops and weeds (farming systems, catch crops or weeds). The root biomass of wheat and barley varied between years from 118 to 199 $g\ m^{-2}$; however, there was consistently higher root biomass in wheat compared with barley (Tables 5 and 7). The difference in cereal root biomass between organic and conventional farming was 79 $g\ m^{-2}$ (Table 5) and 58 $g\ m^{-2}$ (Table 7). Considering the small difference between the arithmetic means (Table 5) and the least square means (Table 7) for catch crops and weeds, the unbalanced data collected did not appear to have caused much difference to the estimated root biomass.

4. Discussion

4.1. Factors affecting root biomass

Root biomass of cereal crops, catch crops and weeds was affected by both environmental and management factors (Tables 4 and 6). The results showed significant effects of year, species and farming systems on root biomass in cereal crops. For catch crops and weeds, significant differences in root biomass were observed between catch crops and weeds and also between organic and conventional farming systems. We acknowledge the existence of confounding data, which with imbalanced data could lead to biased estimates of influential factors on root biomass. However, the analyses clearly pointed to differences in root biomass between farming systems, where data from the same site and year was included for both farming systems.

The reason for the observed factors influencing root biomass may be found in how photosynthesized products are allocated between shoots and roots. During the growing period, shoots and roots interact closely to allocate the photosynthesized material from shoots and the absorbed nutrients from roots (Thornley, 1972). The relative allocation between shoots and roots changes over time in response to the relative need of photosynthesized material and nutrients (Thornley, 1972; Poorter and Nagel, 2000). Less supply of below-ground resources (e.g. nutrients and water) would induce allocation of more photosynthates to roots, while less aboveground resources (e.g. less light) could cause more allocation to shoots (Thornley, 1972; Poorter and Nagel, 2000). Thus for any given species, it is the environment and the soil conditions that determines how much can be photosynthesized and how much is allocated to shoots or roots. The ratio between shoot and root biomass is therefore the result of changing allocation patterns during the growing period. The dynamic association between shoots and roots means that allometric ratios are not well suited for calculating root biomass, since the final allometric ratios can be quite variable, especially under stressed environmental and soil nutritional conditions.

Environmental conditions (e.g., radiation, precipitation and temperature) varied between the experimental years (Table 3). Therefore, the total carbon assimilation, the fraction allocated to roots and root distributions within the soil profile could also differ between years. In our data, the lowest root biomass for cereals in 0–25 cm was observed in 2013, whereas a higher level of root biomass was found for the other years. The spring of 2013 was characterized by drier conditions than for the other years, which may have caused plants to develop deeper roots and less dense roots in the upper soil layer in 2013. This was also indicated by the observed root biomass (data not shown) that showed less difference in root biomass between 2013 and 2014 for the depth 0–60 cm than for 0–20 cm. Genotypic variation between species could cause different specific allocation strategies (Fakhri et al., 1987; Clark et al., 2003), and thus cause root biomass differences among species. From the aspects of species, catch crops had higher biomass than weeds, because catch crop species were chosen to fit the growing conditions after main crops (Snapp et al., 2005).

As to farming systems, nutrients, especially nitrogen, in organic farming are less readily available, even though the total input is not always less than in the conventional systems (Stockdale et al., 2002). This lower availability of nutrients is one of the major causes of relatively higher allocation of photosynthates to roots (Poorter and Nagel, 2000; Lonhienne et al., 2014).

4.2. Differences between root biomass estimation methods

The main objective of this work was to compare root biomass estimation methods, particularly the use of fixed allometric relations versus fixed root biomass. The results showed that using fixed root biomass based on the most influential factors provided the most robust estimation with MBE_P close to 0, and generally the lowest $RMSE_P$. Using allometric relations for estimating root biomass resulted in higher MBE_P and $RMSE_P$ than using fixed root biomass, in terms of both most influential factors and commonly used factors (factors in brackets in Tables 4 and 6). Generally, shoot/root ratios provided negative MBE_P and lower $RMSE_P$ than other ratios. Shoot/all or root/all ratios generally provided positive MBE_P and higher $RMSE_P$. Root/shoot ratios generally had a higher positive MBE_P and the highest $RMSE_P$.

As discussed above, root biomass of a certain species depends on environmental and management factors. A robust and unbiased estimate of root biomass requires that the MBE_P is close to zero and the $RMSE_P$ from cross validation is as small as possible. In root/shoot, shoot/root, root/(shoot + root) or shoot/(root + shoot) ratios, either one part (shoot or root) or the total biomass appears as the denominator. The allometric ratios for individual measures may vary greatly due to the variation in either above- or belowground biomass, which may cause biases in the estimation of the mean allometric ratio. Furthermore, with allometric ratios root biomass will be estimated only from observed shoot biomass, and any uncertainty in observed shoot biomass will be translated to uncertainty in root biomass amplified by the uncertainty in the allometric relationship.

Generally, we observed the following relations between organic farming and conventional farming: 1) more shoot biomass associated with less root biomass was found in conventional farming, and the opposite in organic farming; 2) more total (shoot + root) biomass associated with less root biomass in conventional farming, and a relatively more equal distribution between shoots and roots in organic farming; 3) the difference in root biomass between the two farming systems (highest root biomass in organic farming) is generally smaller than that in shoot biomass (highest shoot biomass in conventional farming). If we estimated root biomass with the existence of all these three relations, root biomass would be highly overestimated when using root/shoot ratios, less underestimated when using shoot/root ratios, and less overestimated using root/(root + shoot) ratios. Thus, the highly dynamic relations between shoot and root biomass is affected by the type of farming systems as well as by the actual management. Therefore, root biomass can for the climatic conditions of northern Europe more reliably be estimated using fixed values depending on farming system and plant species rather than assuming a dependency on shoot biomass.

4.3. Perspectives

Our results from Denmark show that the most practical and accurate estimates of root biomass are obtained by using fixed root amounts that depend on farming system and species (Table 5). It would be valuable to have similar analyses for other climatic and soil conditions, and for other types of farming systems. From our results, considering only farming systems for cereals would give almost similar performance. For catch crops and weeds separate fixed values should be used to provide the best estimates. The observed differences in root biomass between years, especially in the upper soil layer, indicate that robust root biomass estimates should be based on measurements over several years.

Most studies on root biomass in cereals have been conducted in conventional farming systems, and our estimates of root biomass generally agree with findings from other studies in northern Europe. As corrected by Eq. (1) to a depth of 0–25 cm: Van Noordwijk et al. (1994) in the Netherlands measured root of winter wheat as 133–154 $g\ m^{-2}$; Kätterer et al. (1993) reported winter wheat root biomass in Sweden of 79–90 $g\ m^{-2}$; Braim et al.(1992) reported barley root biomass in Britain of around 107–116 $g\ m^{-2}$; Pietola and Alakukku (2005) reported root biomass for barley and oats at anthesis in Finland of 98 and 215 $g\ m^{-2}$, respectively; Głąb et al. (2014) reported triticale root biomass of 94–160 $g\ m^{-2}$. These values are comparable with our results of $142 \pm 30\ g\ m^{-2}$ for wheat and $129 \pm 19\ g\ m^{-2}$ for barley.

In other parts of the world, we would also recommend use of fixed root amounts for estimation for root biomass, because estimated root biomass with allometric ratios from our results are not only inaccurate, but also biased (Tables 4 and 6). However, there are also limitations for fixed root estimation, because roots are inadequately sampled across the world. Therefore, in cases where no root biomass observations are available and where climate and soil conditions differ substantially from reference sites, the use of allometric ratios may become inevitable. In such situations, we would recommend use of shoot to root ratio for root biomass estimation (Tables 4 and 6), even though shoot to root ratio may induce underestimation of root biomass. In any case, our results clearly point to the need for improving the globally available data on root biomass, and ideally these data should be made available in an open repository for use by both experimentalists and modellers.

Soil carbon sequestration plays a potential role in mitigation of climate change and root biomass contributes with a significant carbon input (Gattinger et al., 2012). Our results indicate that roots in organic farming systems may contribute more to soil carbon sequestration than in conventional systems. Taghizadeh-Toosi et al. (2016) similarly reported that the root carbon input can be considered constant across different nitrogen fertiliser rates. The estimates of fixed root amount (Table 5) can be used to improve calculations of belowground carbon input in modelling. Assuming the percentage of carbon in roots as 45% (Chirinda et al., 2012), organic farming would then bring in roughly 0.6 Mg ha^{-1} more C input than conventional farming from both cereals and catch crops.

5. Conclusions

A statistical analysis of root biomass data from field experiments in Denmark showed that the use of fixed root biomass provided lower error of prediction for estimation of root biomass than the use of fixed allometric ratios. The most robust estimation of root biomass was found with fixed root biomass depending on farming system and plant type. However, there was some variation between years in root biomass of cereals. There was consistently greater root biomass of cereal crops in organic compared to conventional systems, and there was greater root biomass in wheat compared to barley. The results also showed greater root biomass in catch crops compared with weeds.

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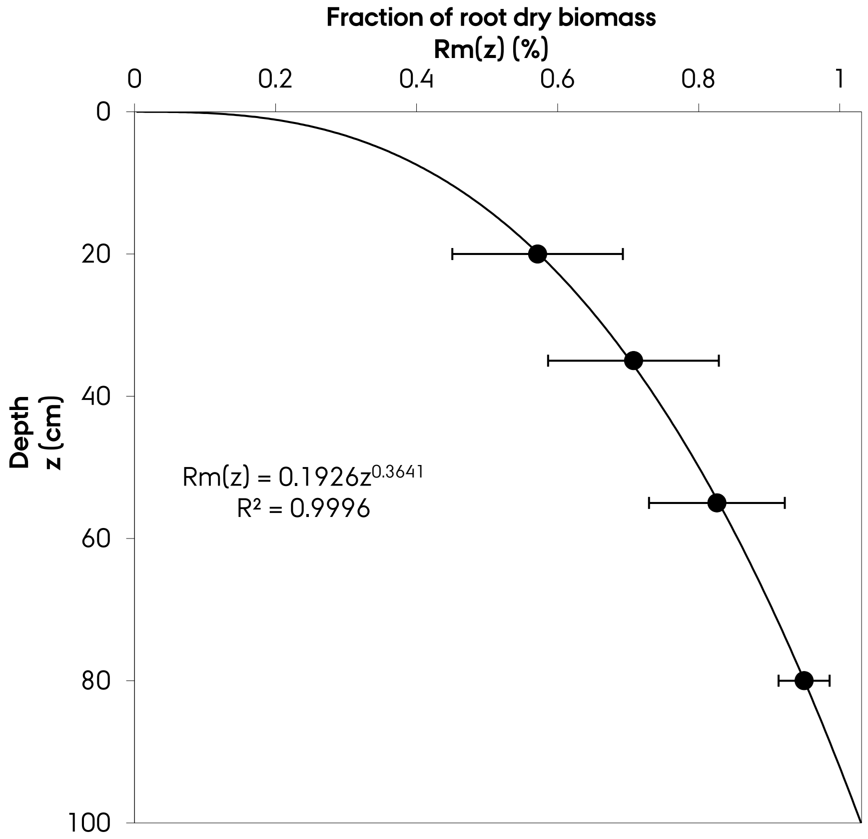

Fig. S1. Relative distributionof fodder radish root dry matter within 100 cm depth measured in November at Flakkebjerg in Denmark. Bars indicate the standard error (n=12) (Wahlström et al., unpublished).

Table S1. Impacts of farming systems and N application on vertical root biomass distribution of cereals, catch crops and weeds.

Year	Fraction of root biomass distribution	Management	Spring Barley	Winter wheat	Catch crops and weeds
2008	(0-30 cm)/(0-60 cm)	Organic	0.85 (0.02)	0.91 (0.02)	0.73 (0.06)
		Conventional	0.82 (0.03)	0.92 (0.01)	0.67 (0.07)
2013	(0-20 cm)/(0-60 cm)	Low N		0.67 (0.06)	
		High N		0.63 (0.08)	
2014	(0-20 cm)/(0-60 cm)	Low N		0.84 (0.04)	
		High N		0.82 (0.04)	

Values in brackets show the standard error.

Paper II

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Soil carbon varies between different organic and conventional management schemes in arable agriculture

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ABSTRACT

The effects of organic versus conventional farming systems on changes in soil organic carbon (SOC) has long been debated. The effects of such comparisons may depend considerably on the design of the respective systems and climate and soil conditions under which they are performed. Here, we compare a range of arable organic and conventional crop systems at three sites (Jyndevad, Foulum and Flakkebjerg) in Denmark through long-term experiments initiated in 1997. The experimental treatments in the organic farming systems included use of whole-year green manure crops, catch crops and animal manure (as cattle, pig or digested slurry). Data on plant residues and animal manure were used to estimate C inputs to the soil. This was compared with measured changes in topsoil (0–25 cm) SOC content over 4–8 years.

During 1997–2004, green manure, catch crops and animal manure enhanced estimated C input by 0.9, 1.0 and 0.7 Mg C ha^{-1} yr^{-1} respectively, across all locations. Based on measured SOC changes, green manure enhanced SOC by 0.4 Mg C ha^{-1} yr^{-1} and catch crops by 0.2 Mg C ha^{-1} yr^{-1}, while animal manure by insignificantly 0.1 Mg C ha^{-1} yr^{-1}. After 2005, advantages of using green manure (grass-clover) on SOC change disappeared, because cuttings of the grass-clover was removed whereas before 2005 they were mulched in the field, albeit there was still a small extra estimated C input of 0.2 Mg C ha^{-1} yr^{-1}. An estimated higher C input of 0.7 Mg C ha^{-1} yr^{-1} with catch crops did not result in significant increase in measured topsoil SOC.

From 2005–2008, the first 4 years of comparison between organic and conventional farming at all three sites, organic farming with animal manure had 0.3 Mg C ha^{-1} yr^{-1} higher estimated C input, but SOC measurements showed that conventional farming accumulated 0.4 Mg C ha^{-1} yr^{-1} more SOC than organic farming. At Foulum from 2005 to 2012, organic farming with animal manure had 0.7 Mg C ha^{-1} yr^{-1} more input, and topsoil SOC measurements showed a higher accumulation of 0.4 Mg C ha^{-1} yr^{-1} in organic compared with conventional farming.

Regressions of changes in topsoil SOC against estimated C inputs showed that 10–20% of C inputs were retained in topsoil SOC over the experimental period. There was no clear indication that belowground C input contributed more to SOC than aboveground C inputs. Despite consistently higher estimated C inputs in organic versus conventional systems, we were not able to detect consistent differences in measured SOC between the systems.

1. Introduction

Globally, soil is one of the most important terrestrial stores of carbon (C) (Davidson et al., 2000; Lal, 2008; Lehmann and Kleber, 2015); however, agricultural soil C is undergoing substantial change due to both environmental conditions and management effects (Janzen et al., 1997; West and Post, 2002; Crowther et al., 2016). Soil organic C (SOC) is an essential indicator of soil fertility and soil quality (Susanne and Michelle, 1998; Al-Kaisi et al., 2005; Huang et al., 2007; Merante et al., 2017). Properly managing SOC may not only bring benefit to productivity and environment, but also mitigate negative effects of extreme events, like droughts, by improving soil hydraulic properties (Gomiero et al., 2011). Enhancing SOC can contribute to reducing net agricultural greenhouse gas emissions, not only by storing C in soils, but also facilitating changes in soil structure that in some cases may reduce N_2O emissions (Mutegi et al., 2010; Powlson et al., 2011). SOC is also associated with higher contents of nutrients such as nitrogen, phosphorus and sulphur (Kirkby et al., 2011), and managing SOC is therefore also closely linked to soil nutrient management, in particular in organic farming (Watson et al., 2002; Gomiero et al., 2011; Reganold

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Table 1
Structure of the organic (O) and conventional (C) crop rotations at three locations: JY = Jyndevad, FO = Foulum, FL = Flakkebjerg.

Crop rotations	O1			O2			O4			C4		
Cycles	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]	Crop	M[1]	CC[2]
1st cycle 1997–2000	S. barley:ley	50		S. barley:ley	50		S. oat	40	+[5]			
	Grass-clover	0		Grass-clover	0		W. wheat	70	+[5]			
	S. wheat	50	+[3]	W. wheat	50	+[3]	W. cereal	70	+[5]			
	Lupin	0	+[4]	Pea/barley	0	+[4]	Pea/barley	0	+[4]			
2nd cycle 2001–2004	S. barley:ley	50		S. barley:ley	50		W. wheat	50	+[4]			
	Grass-clover	0		Grass-clover	0		S. oat	50	+[4]			
	S. oat	30	+[3]	W. cereal	50	+[3]	S. barley	50	+[3]			
	Pea/barley	0	+[4]	Lupin	0	+[4]	Lupin	0				
Locations	JY			JY, FO, FL			FO, FL					
3rd cycle 2005–2009	Discontinued			S. barley:ley	60		S. barley	60	+[4]	S. barley	130	+[3]
				Grass-clover	0		F. bean	0	+[4]	F. bean	0	+[3]
				Potato	100		Potato	110		Potato	140	
				W. wheat	100	+[4]	W. wheat	110	+[4]	W. wheat	165	+[3]
Locations				JY, FO, FL			JY, FO, FL			JY, FO, FL		
4th cycle 2010–2012				S. barley:ley	60		S. barley	60	+[4]	S. barley	120	+[3]
				Lucerne, 1st	0		Hemp	90		Hemp	125	
				Lucerne, 2nd	0		Peas/barley	0	+[4]	Pea/barley	0	+[3]
				S. wheat	100	+[4]	S. wheat	100	+[4]	S. wheat	110	+[3]
				Potato	100	+[4]	Potato	100	+[4]	Potato	140	+[3]
Locations				FO			FO			FO		

[1]M: Manure application target rates in +M treatments. Unit: kg NH_4-N ha^{-1} in 1st and 2nd cycles and kg total-N ha^{-1} in 3rd cycle. Inorganic fertilizer rates are shown as target mineral N in kg N ha^{-1}. [2]CC: Crops succeeded by catch crops in +CC treatments. [3]Monocultures or mixtures of non-N_2-fixing catch crop. [4]Mixtures of N_2-fixing and non-N_2-fixing catch crop. [5]White clover.

and Wachter, 2016).

SOC is primarily managed through soil C inputs, since tillage intensity has shown to have little effect on total SOC storage, although the vertical profile of C concentration is affected by tillage (Powlson et al., 2014). Enhancing SOC thus requires that additional C is added to the soil, which may be achieved by enhancing crop productivity to achieve a higher amount of crop residues or by retaining a larger proportion of the residues in the cropping systems (Powlson et al., 2011). Organic farming, as an approach to environmentally friendly agriculture practice (Reganold and Wachter, 2016), emphasizes increasing SOC and enhancing nutrient cycling through measures such as growing green manure and catch crops, and applying manure (Olesen et al., 2007), which provides additional sources of C inputs besides residues from arable crops. Organic farming has been demonstrated to have higher total C input (Gattinger et al., 2013) and topsoil SOC stocks (Gomiero et al., 2011; Gattinger et al., 2012; Tuomisto et al., 2012) than conventional farming. This is partly a consequence of higher external C input in organic farming, e.g. through animal manure and compost. Compared to conventional farming, organic farming has been criticised for lower crop yields that may lead to lower C inputs (Connor, 2008; Leifeld, 2012; Seufert et al., 2012) and less net transfer of C to the soil from photosynthesis of the crops being grown (Leifeld et al., 2013). However, crops vary greatly in their C inputs from above- and belowground crop residues, and in particular, belowground C inputs are difficult to quantify. Recent research strongly suggests that belowground C input is independent of aboveground biomass for many crop species (Chirinda et al., 2012; Taghizadeh-Toosi et al., 2016; Hu et al., 2018). Additionally, higher root biomass C input of cereals in organic farming compared to conventional systems indicates that belowground C input in organic farming systems may be underestimated (Chirinda et al., 2012). The inputs of C from roots and rhizodeposition may be of particular importance for SOC, since studies have shown that these sources of C may be better retained in soils than C from aboveground crop residues (Rasse et al., 2005; Kätterer et al., 2011; Berti et al., 2016).

There is thus a need to improve the understanding of how the management measures in organic farming contribute to C inputs and retention in soils. Data from long-term experiments with variation in cropping system design and crop management may provide valuable insights by providing information on C inputs and on changes in SOC storage. Such long-term experiments were initiated at three sites in Denmark in 1997 (Olesen et al., 2000), and they thus provide an opportunity to reveal how different components of organic farming systems contribute to soil C inputs and to changes in SOC. The aim of this study was to assess how different components from conventional and organic cropping systems in long-term experiments in Denmark contribute to changes in SOC. For this, we hypothesize: 1) Green manure crops, catch crops and manure add significant amounts of C to the soil that also contribute to measureable changes in SOC; 2) Organic farming can provide higher C input than conventional farming, and this will result in higher SOC of organic compared with conventional farming; 3) Belowground plant inputs contribute to SOC through higher retention of the added organic C than for aboveground parts.

2. Materials and methods

2.1. Field sites

Changes in soil C monitored in long-term experiments on organic and conventional cropping systems at three sites in Denmark, varying in soil type and climate, i.e. Jyndevad (54°54′N, 09°08′E), Foulum (56°30′N, 09°35′E) and Flakkebjerg (55°20′N, 11°23′E) were used for this study. Jyndevad is located in Southern Jutland on a coarse sandy soil (Gleyic Podzol), Foulum is situated in Central Jutland on loamy sand soil (Mollic Luvisol), and Flakkebjerg is placed in Western Zealand on sandy loam soil (Glossic Phaeozem) (classification according to WRB and FAO). In the topsoil (0–25 cm), the clay content at Jyndevad, Foulum and Flakkebjerg were 45, 88 and 155 g kg^{-1}, respectively. The soil pH of the respective locations was 6.1, 6.5 and 7.4. SOC content was 1.17, 2.29 and 1.01%, and soil C/N ratio was 13.8, 13.1 and 9.4 at start of the experiments. The soil bulk density at Jyndevad, Foulum and Flakkebjerg were 1.572, 1.422 and 1.702 $g\,cm^{-3}$. Average annual temperature and precipitation during 1961–1990 were 7.9 °C and 964 mm, 7.3 °C and 704 mm and 7.8 °C and 626 mm for the three sites, respectively. Additional information on soil properties of these three locations are provided by Olesen et al. (2000) and Berntsen et al. (2004).

2.2. Experimental treatments

The experiments were conducted according to an experimental design with three factors. At all three sites, four or five-year crop rotation cycles were used, where four crops in the rotations were present every year (Table 1). From 1997–2004, three factors were included in a fully factorial design: (i) N_2-fixing whole-year green manure crops in organically managed rotations (with: O1 and O2, without: O4), (ii) catch crops (with: +CC, without: −CC), and (iii) manure (with: +M, without: −M), which composed 8 treatment combinations. During this period, O1 was conducted at Jyndevad, whereas O4 was conducted at Foulum and Flakkebjerg. From 2005, rotation O4 replaced O1 at Jyndevad (Askegaard et al., 2011). Since 2005, treatments of O2-CC-M and O4-CC-M were converted to conventional rotation (without N_2-fixing green manures, but using inorganic fertilisers: +IF), as C4-CC+IF and C4+CC+IF, in which mineral fertilisers and pesticides were used (Shah et al., 2017). The experiments were conducted at all three locations until 2009, when it was stopped at Jyndevad and Flakkebjerg, but continued at Foulum. To obtain better control of perennial weeds (*Cirsium arvense* L. and *Elytrigia repens* L.) the crop rotations were converted in 2010 from 4 to 5 years. In particular, an additional year of green manure was added in O2, while hemp was introduced in O4 and C4. During the original experiment design all four crops in the rotation was represented every year in each treatment, but from 2010 only 4 of the 5 crops were present in any given year. The experiments were conducted with 2 replicates in a total of 64 plots at each site every year. Since the analyses were done on 4-year rotational basis, there were in total 8 replications for each of the 8 aforementioned treatment combinations. Plots sizes were 378, 216 and 169 m^2 at Jyndevad, Foulum and Flakkebjerg, respectively.

2.3. Crop management

The main crops included in the experiment were: spring barley (*Hordeum vulgare* L.), spring and winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.), winter triticale (*Triticosecale*), lupin (*Lupinus angustifolius* L.), faba bean (*Vicia faba* L.), a mixture of pea (*Pisum sativum* L.) and spring barley, potato (*Solanum tuberosum* L.), grass-clover, mainly including perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) (Table 1).

In the first two 4-year cycles (1997–2004), the non-legume catch crops varied between monocultures of ryegrass or mixtures of ryegrass and chicory (*Cichorium intybus* L.) undersown in spring. The legume catch crop varied between pure stands of white clover, mixtures of ryegrass + white clover or mixtures of ryegrass + white clover + red clover or mixtures of ryegrass + black medic (*Medicago lupulina* L.) + serradella (*Ornithopus sativus Brot.*) + birdsfoot-trefoil (*Lotus corniculatus* L.) + subterranean clover (*Trifolium subterraneum* L.) or a mixture of ryegrass + chicory + black medic + kidney vetch (*Anthyllis vulneraria* L.). All catch crop mixtures were undersown in spring. From the 3rd cycle (2005–2009), a mixture of winter rye + hairy vetch (*Vicia villosa* L.) + fodder radish (*Raphanus sativus oleiformis* L.) sown after harvest of the crop was used at Flakkebjerg. From the 4th cycle (2010–2012), mixtures of radish + rye, radish + rye + vetch or chicory + grass + clover were used at Foulum. Various catch crop species were chosen over time reflecting experience of which species provided the most reliable establishment and growth.

From 1997–2004, in treatments with manure (+M), cattle slurry, pig slurry and anaerobically digested slurry was used at Jyndevad, Foulum and Flakkebjerg, respectively, at ammonium-N rates corresponding to 40% of the recommended N rates for conventional farming in Denmark (Plantedirektoratet, 1997). From 2005, pig slurry was used at all three locations, and rates applied were updated according to a revised Danish national standard allowing import of animal manure of conventional origin corresponding to 70 kg total-N $ha^{-1}\,yr^{-1}$ (Plantedirektoratet, 2005). From 2011 anaerobically digested slurry was used at Foulum. Analyses of manure N contents confirmed that the actual rates of N applied were close to the target levels.

From 1997–2008, all straw was incorporated into the soil or left on the ground. From 2010 onwards at Foulum, straw of spring barley, spring wheat and peas/barley were removed in C4 treatments. From 2011 onwards, removing straw of spring barley was extended to O2 treatments.

Before 2005, grass-clover was cut 3–4 times and left on the ground in all treatments of rotation O1 and O2 in the growing season, except in 1999 at Jyndevad for controlling couch grass (*Agropyrum repens* L.). Since the 3rd cycle in 2005, the grass-clover cuttings were removed in the +M treatments.

Mechanical weed harrowing (tine harrowing in cereals and pulses and ridging in potatoes) were conducted to control weeds. In −CC treatments harrowing (stubble cultivation) was conducted in autumn when there was a need to control perennial weeds. In some years, +CC treatments identified to have high level of weeds were harrowed immediately after harvest before establishing the catch crops.

In the 1st and 2nd cycles, Jyndevad was the only location irrigated. After introduction of potato in the 3rd cycle, irrigation was conducted in the plots with potato at Flakkebjerg and in all plots at Foulum according to the need for irrigating potato. Irrigation was not applied in the 4th cycle, where the experiment was only conducted at Foulum (Table 1).

2.4. Soil sampling

Soil samples to 25 cm depth in each plot were taken for SOC content measurement in 1996, 2004, and 2008 at all locations and in 2012 at Foulum. In each plot, eight soil samples were taken and pooled to a composite sample. Soil C percentage contents were then measured using a LECO CNS-1000 analyser with IR detector (LECO Corporation, St. Joseph, MI). SOC percentage content was calculated by subtracting the percentage content of carbonates from soil C, if present (Nelson and Sommers, 1996). SOC percentage contents were converted to topsoil SOC amounts by multiplying by 0–25 cm soil bulk densities of each location and the associated soil volume. Soil density was assumed independent of treatments.

2.5. C input estimation

Soil C input originated from green manure, catch crops, crops and animal manure. The aboveground biomass returned to the soil was estimated by measured aboveground biomass minus harvested biomass of each crop and catch crop. Each plot was subdivided into four or five subplots. Two of the subplots were harvested for crop yield. The other subplots were used for plant and soil sampling. The size of the net harvest plots was 22.5, 24 and 16 m^2 at Jyndevad, Foulum and Flakkebjerg, respectively. Cereal and grain legume crops were harvested in August using a combine harvester, whereas potato was harvested with a potato harvester and grass with a plot grass harvester. Aboveground biomass at all plants (potatoes excluded) was sampled from two 0.5 m^2 sampling plots. Crops (potatoes excluded) were sampled shortly before maturity. Potatoes were sampled as 10 plants per plot in organic rotations at the early stage of potato late blight and in conventional rotations before spraying to wilt the crop. Green manures were sampled shortly before each cut. Samples of catch crops were taken during late autumn (about November). The aboveground dry matter (DM) contents of plant samples were weighed after oven drying at 80 °C for 24 h.

Belowground C inputs from roots were based on estimates of root biomass, which was assumed as fixed amounts depending on farming systems (organic or conventional) and species, and thus independent of aboveground biomass (Chirinda et al., 2012; Taghizadeh-Toosi et al., 2016). This assumption was validated based on measurements from a range of studies conducted in Denmark (Hu et al., 2018). Because of similar species and cutting times, first production year data of red clover from Bolinder et al. (2002) was used to estimate the fixed root amount of grass-clover crops. For the green manure crop of lucerne grown after 2010, lucerne data of the first and second production year from Bolinder et al. (2002) were used separately for the first and second production years of lucerne, respectively, in the experiment. Estimates of root biomass of cereals (wheat, barley and their average for other cereals), catch crops and weeds for organic and conventional farming systems were taken from Hu et al. (2018). Legume:barley mixtures were regarded as barley for calculating belowground C input. Fixed root dry biomass of potatoes from 25 to 30 cm depth was collected from Bolinder et al. (2015). Root biomass data for faba bean (Munoz-Romero et al., 2011), lupin (Russell and Fillery, 1996) and hemp (Amaducci et al., 2008) were used to estimate fixed root amounts for these crops.

C content of applied animal manure was measured using a LECO CNS-1000 analyzer. C content in plant material was taken as 0.45 of the dry biomass for residues and roots (Chirinda et al., 2012). Root biomass (except for potato) was corrected to 0–25 cm depth using the Michaelis-Menten-type root depth distribution function of Kätterer et al. (2011). Soil C inputs from roots also include root exudates, which were taken as 0.65 times root biomass C according to Bolinder et al. (2007). Estimation of belowground C inputs for individual crops and farming systems are shown in Table 2.

Table 2
Estimated root + exudates C for different type of plants.

Farming systems	Species	Estimated root + exudates C input $Mg\,C\,ha^{-1}\,yr^{-1}$	Reference
Organic systems	Grass-clover	4.43	Bolinder et al. (2002)
	Lucerne	3.03	Bolinder et al. (2002)
	Lucerne 2nd	5.36	Bolinder et al. (2002)
	Wheat	1.80	Hu et al. (2018)
	Barley	1.43	Hu et al. (2018)
	Other cereals	1.62	Hu et al. (2018)
	Catch crops	0.94	Hu et al. (2018)
	Weeds[1]	0.26	Hu et al. (2018)
Conventional systems	Wheat	1.05	Hu et al. (2018)
	Barley	0.96	Hu et al. (2018)
	Other cereals	1.03	Hu et al. (2018)
	Catch crops	0.56	Hu et al. (2018)
	Weeds[1]	0.21	Hu et al. (2018)
Both systems	Lupin	1.59	Russell and Fillery (1996)
	Potato	0.22	Bolinder et al. (2015)
	Faba bean	1.11	Munoz-Romero et al. (2011)
	Hemp	1.12	Amaducci et al. (2008)

[1] Treatments/years without catch crops and soil tillage in autumn.

2.6. Statistical analysis

Data on total C input and changes in SOC content (0–25 cm) of O2, O4 and C4 at all locations were analysed separately for different periods, i.e. from 1996 to 2004 and from 2005 to 2008. Data from rotation O1 were not included, since this rotation was only conducted at Jyndevad. Data from Foulum were also analysed from 2005 to 2012. This reflected the different design of the systems in the different periods, with conventional systems only being present from 2005 onwards. Effects of treatments (crop rotation, catch crops and manure) on total C input and changes in SOC were analysed using an analysis of variance with the procedure GLM of SAS (SAS Institute, 2008), and the Least Significant Difference (LSD) ($P < 0.05$) was used for the significance of mean observation differences.

The following regression models were used to estimate the contribution of C input in plant materials (aboveground plus belowground), animal manure and original SOC content to the SOC content during 1996–2008 at all locations and 1996–2012 at Foulum:

$$C_t = K_P \times C_{plant} + K_{AM} \times C_{AM} + K_O \times C_o \quad (1)$$

where C_t is the SOC content ($Mg\,C\,ha^{-1}$) at sampling time t (2008 or 2012), C_{plant} is the C input from plant material ($Mg\,C\,ha^{-1}$), C_{AM} is C input in animal manure ($Mg\,C\,ha^{-1}$), and C_o is the SOC content ($Mg\,C\,ha^{-1}$) of soil sampled in topsoil (0–25 cm) at the starting time (in 1996). K_P and K_{AM} are coefficients describing the effects of C inputs from plant materials and animal manure, respectively. K_O defines the effect of original SOC content on C_t at sampling time t.

Effects of aboveground parts and belowground parts on the SOC content were examined with the following model:

$$C_t = K_A \times C_{aboveground} + K_B \times C_{belowground} + K_{AM} \times C_{AM} + K_O \times C_o \quad (2)$$

where $C_{aboveground}$ is the C input in aboveground residues ($Mg\,C\,ha^{-1}$), $C_{belowgorund}$ is C input in roots and rhizodeposition ($Mg\,C\,ha^{-1}$). K_A, K_B and K_{AM} are coefficients describing the effects of the above- and belowground plant C and animal manure inputs, respectively.

The parameters in Eqs. (1) and (2) were estimated using the MIXED procedure of SAS (SAS Institute, 2008), where intercept was set as 0, and block effects nested within locations were set as random. Data in rotation O1 were also used in modelling for these equations. In Eqs. (1)–(2), K_O refers to the proportion of original SOC left in soil. Thus, the proportion of C lost every year is calculated as:

$$D_C = 1 - K_O^{\,1/n} \quad (3)$$

Where D_C means proportion of SOC decomposed in one year (decomposition rate of original SOC), and n is the span of years.

3. Results

3.1. C input

3.1.1. All sites, 1997–2004

Across all three sites from 1997 to 2004, mean C inputs were around 3.62–6.62 $Mg\,C\,ha^{-1}\,yr^{-1}$ (Table 3). The highest C inputs were observed in O2+CC+M (Jyndevad and Flakkebjerg) and O4+CC+M (Foulum). The lowest C inputs consistently occurred in O4-CC-M (Table 3). As shown in Fig. 1a for average C inputs, the one-year green manure provided approximately 2.22 $Mg\,C\,ha^{-1}\,yr^{-1}$ of the 5.71 $Mg\,C\,ha^{-1}\,yr^{-1}$ for the entire 4-year rotation as average of the O2 treatments. Crops provided around 2.89 $Mg\,C\,ha^{-1}\,yr^{-1}$ in O2 and 3.85 $Mg\,C\,ha^{-1}\,yr^{-1}$ in O4. C inputs from catch crops, if applied, provided about 0.77 $Mg\,C\,ha^{-1}\,yr^{-1}$ in O2 and 1.67 $Mg\,C\,ha^{-1}\,yr^{-1}$ in O4, while weeds supplied around 0.28 $Mg\,C\,ha^{-1}\,yr^{-1}$. Animal manure added another 0.23 $Mg\,C\,ha^{-1}\,yr^{-1}$ of C input. Significant treatment effects (green manure, catch crops and animal manure) on C inputs

Table 3

Mean carbon input (Mg C ha^{-1} yr^{-1}) estimated in different treatments at Jyndevad, Foulum, Flakkebjerg during 1997–2004 (Jyndevad, Foulum and Flakkebjerg), 2005–2008 (Jyndevad, Foulum, Flakkebjerg) and 2005–2012 (Foulum). Analysis was also conducted with data of all three locations combined during 1997–2004 and 2005–2008. Part I of the table shows carbon inputs for individual treatment components and part II shows main effects of individual treatments. In part II treatments with –M were not used in analysis after 2004.

Treatment	Jyndevad		Foulum			Flakkebjerg		All locations	
	1997–2004	2005–2008	1997–2004	2005–2008	2005–2012	1997–2004	2005–2008	1997–2004	2005–2008
Part I									
O2-CC-M	4.43 ^{c}	–	5.48 ^{e}	–	–	5.57 ^{c}	–	5.16 ^{c}	–
O2-CC + M	5.33 ^{b}	3.62 ^{bc}	6.02 ^{cd}	4.46 ^{c}	4.19 ^{c}	6.01 ^{b}	3.64 ^{cd}	5.79 ^{b}	3.91 ^{c}
O2 + CC-M	4.96 ^{b}	4.44 ^{a}	6.18 ^{bc}	5.41 ^{a}	5.48 ^{a}	6.13 ^{b}	4.45 ^{a}	5.76 ^{b}	4.77 ^{a}
O2 + CC + M	5.75 ^{a}	4.32 ^{a}	6.32 ^{b}	4.72 ^{b}	4.58 ^{b}	6.36 ^{a}	4.15 ^{ab}	6.14 ^{a}	4.39 ^{b}
O4-CC-M	–	–	3.93 ^{g}	–	–	3.62 ^{e}	–	3.48 ^{e}	–
O4-CC + M	–	3.30 ^{d}	4.77 ^{f}	3.63 ^{e}	3.50 ^{d}	4.60 ^{d}	3.11 ^{ef}	4.39 ^{d}	3.35 ^{e}
O4 + CC-M	–	3.61 ^{bc}	5.80 ^{d}	4.20 ^{d}	4.17 ^{c}	5.34 ^{c}	3.38 ^{de}	5.27 ^{c}	3.73 ^{d}
O4 + CC + M	–	4.48 ^{a}	6.62 ^{a}	4.89 ^{b}	4.74 ^{b}	6.20 ^{ab}	4.12 ^{b}	6.11 ^{a}	4.50 ^{b}
C4-CC + IF	–	3.33 ^{cd}	–	3.61 ^{e}	3.15 ^{e}	–	3.01 ^{f}	–	3.32 ^{e}
C4 + CC + IF	–	3.71 ^{b}	–	4.06 ^{d}	3.66 ^{d}	–	3.72 ^{c}	–	3.83 ^{cd}
Part II									
O2	–	3.97 ^{a}	6.00 ^{a}	4.59 ^{a}	4.39 ^{a}	6.02 ^{a}	3.90 ^{a}	5.71 ^{a}	4.15 ^{a}
O4	–	3.89 ^{a}	5.28 ^{b}	4.26 ^{b}	4.12 ^{b}	4.94 ^{b}	3.62 ^{b}	4.81 ^{b}	3.92 ^{b}
C4	–	3.52 ^{b}	–	3.84 ^{c}	3.41 ^{c}	–	3.36 ^{c}	–	3.57 ^{c}
-CC	4.88 ^{b}	3.42 ^{b}	5.05 ^{b}	3.90 ^{b}	3.62 ^{b}	4.95 ^{b}	3.25 ^{b}	4.77 ^{b}	3.52 ^{b}
+CC	5.36 ^{a}	4.17 ^{a}	6.23 ^{a}	4.55 ^{a}	4.33 ^{a}	6.01 ^{a}	4.00 ^{a}	5.76 ^{a}	4.24 ^{a}
-M	4.70 ^{b}	–	5.35 ^{b}	–	–	5.17 ^{b}	–	4.94 ^{b}	–
+M	5.54 ^{a}	–	5.93 ^{a}	–	–	5.79 ^{a}	–	5.59 ^{a}	–

For each group (treatments in Part I; rotations, catch crops and manure respectively in Part II) mean values having different letters within a column are significantly different at the 0.05 significance level.

were observed at all sites (Table 3). Overall, applying green manure increased C input in O2 by 0.90 Mg C ha^{-1} yr^{-1} above that in O4. Using catch crops brought in about 0.99 Mg C ha^{-1} yr^{-1} more C input than weeds (Table 3), where this amount of C input was mainly from catch crops themselves, and partly contributed by higher crop biomass (Fig. 1a). Using animal manure, resulted in additional 0.65 Mg C ha^{-1} yr^{-1} of C input (Table 3). Besides the 0.23 Mg C ha^{-1} yr^{-1} in animal manure itself, the input of 0.41 Mg C ha^{-1} yr^{-1} (0.24 Mg C ha^{-1} yr^{-1} in O2 and 0.59 Mg C ha^{-1} yr^{-1} in O4) was from increased crop biomass due to manure application (Fig. 1a).

3.1.2. All sites, 2005–2008

During 2005–2008, treatments of −CC-M were converted to conventional farming systems where mineral fertilisers were applied. C inputs across all sites ranged from 3.01–5.41 Mg C ha^{-1} yr^{-1} (Table 3). Since the aboveground part of green manure was removed in +M treatments, highest C inputs appeared in O2+CC-M (Foulum and Flakkebjerg) and O4+CC+M (Jyndevad), while the lowest were in C4-CC+IF (Foulum and Flakkebjerg) and O4-CC-M (Jyndevad) (Table 3). As shown in Fig. 1b, green manure brought in 2.42 Mg C ha^{-1} yr^{-1}, but this value dropped to only 1.38 Mg C ha^{-1} yr^{-1} if the aboveground parts were removed. Crops provided around 2.14, 2.89 and 3.16 Mg C ha^{-1} yr^{-1} in O2, O4 and C4, respectively. Accordingly, catch crops contributed about 0.43, 1.11 and 0.69 Mg C ha^{-1} yr^{-1} in each rotation system. In treatments without catch crops, weeds added on average 0.12 Mg C ha^{-1} yr^{-1}. Animal manure itself brought in 0.29 Mg C ha^{-1} yr^{-1}. Generally at all sites, there were significant treatment effects (rotation systems and catch crops) when considering the six fertilised treatments (Table 3). When comparing O2 with O4, using green manure only increased average inputs by 0.23 Mg C ha^{-1} yr^{-1} (Table 3), because the aboveground part of green manure was removed in O2 + M treatments. The aboveground in O2 contributed negatively 0.33 Mg C ha^{-1} yr^{-1} and belowground positively more than 0.56 Mg C ha^{-1} yr^{-1}, compared with O4 (Fig. 1b). Compared to C4, O4 had 0.35 Mg C ha^{-1} yr^{-1} more C input (Table 3), where the belowground part added 0.47 Mg C ha^{-1} yr^{-1} (Fig. 1b), and the aboveground part reduced this value. Applying catch crops increased C inputs with 0.71 Mg C ha^{-1} yr^{-1} (Table 3), where 0.61 Mg C ha^{-1} yr^{-1} of C input was from catch crops, and the rest was from higher biomass yield from the main crops in organic systems, especially in O4.

3.1.3. Foulum, 2005–2012

Even though cereal straw in C4 and barley straw in O2 were removed since 2010 and 2011, the treatment differences of C inputs at Foulum during 2005–2012 did not change much compared to 2005–2008 (Table 3). O2 + CC-M and C4-CC + IF were still the ones that contributed most and least C input, respectively (Table 3). Fig. 1c shows that during the 8 years from 2005 to 2012 at Foulum, green manure provided C inputs of respectively 2.97 and 1.58 Mg C ha^{-1} yr^{-1} without and with removal of grass-clover and lucerne cuts. On average, crops in O2, O4 and C4 brought in 2.15, 3.07 and 2.88 Mg C ha^{-1} yr^{-1}, respectively. Catch crops increase C inputs of 0.53, 1.23 and 0.78 Mg C ha^{-1} yr^{-1} for the respective rotation systems. Weeds and animal manure contributed C inputs of 0.19 and 0.28 Mg C ha^{-1} yr^{-1}, respectively. Green manure with removal of cuttings in O2 helped C input to increase by 0.27 Mg C ha^{-1} yr^{-1} compared to O4 (Table 3), which originated mostly from a higher belowground C input in O2. The C input was 0.71 Mg C ha^{-1} yr^{-1} higher in O4 than in C4, which resulted from differences in C inputs in belowground plant materials and animal manure (Fig. 1c). Catch crops added extra C input of 0.71 Mg C ha^{-1} yr^{-1} (Table 3), which was mostly direct input from the catch crops and less from higher main crop biomass returns.

3.2. Change in SOC

3.2.1. All sites, 1997–2004

From 1997–2004, O2+CC+M at all sites had the highest SOC increase or the least loss, even though the amount of SOC change in the treatment at each site was very different, with 0.40 Mg C ha^{-1} yr^{-1} at Jyndevad, −0.44 Mg C ha^{-1} yr^{-1} at Foulum and 0.27 Mg C ha^{-1} yr^{-1} at Flakkebjerg (Table 4). Generally, the largest decreases in C concentration were obtained with O4-CC-M, except at Jyndevad where O4 was not represented (Table 4). Overall, including the green manure in O2 significantly enhanced SOC by 0.40 Mg C ha^{-1} yr^{-1} when

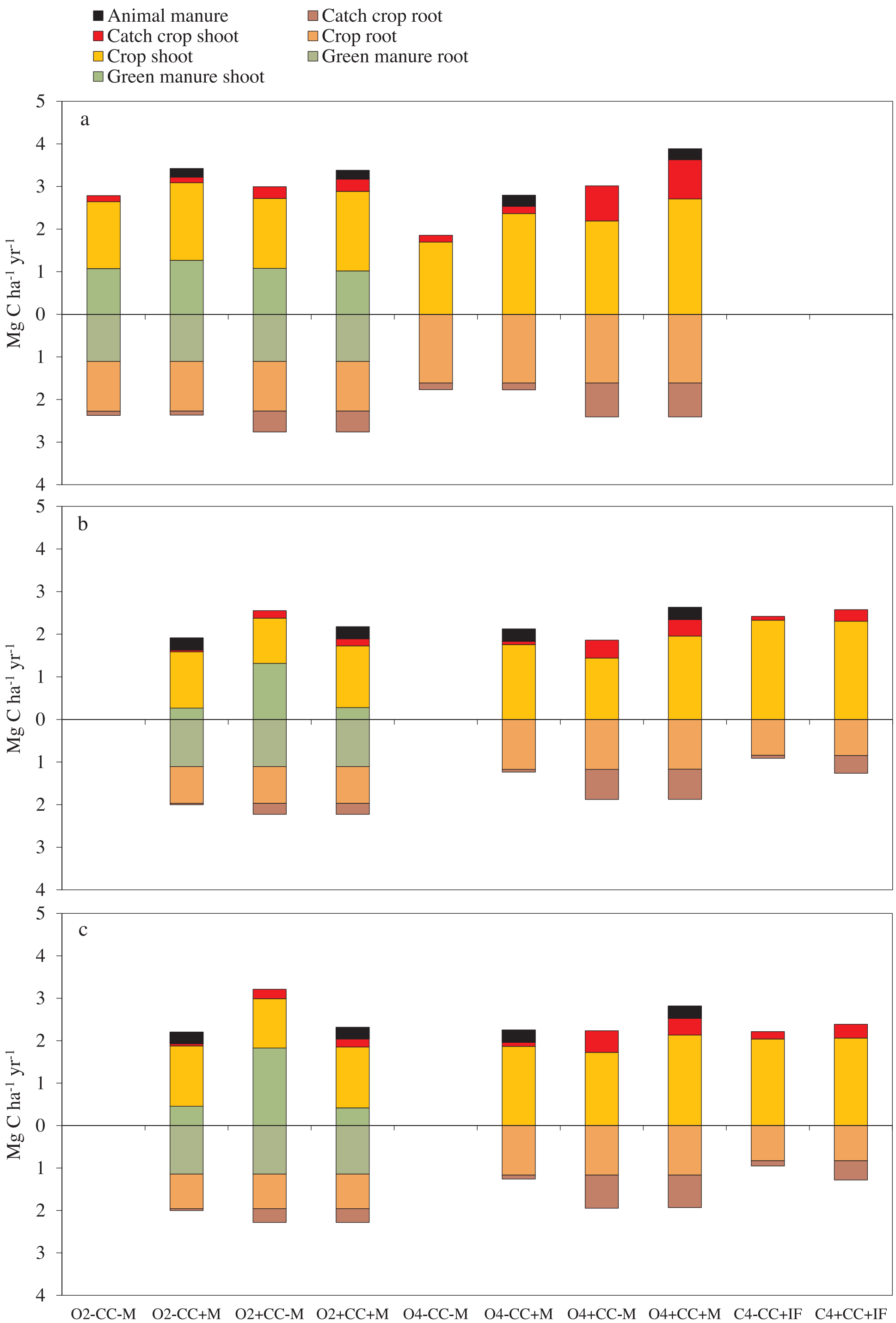

Fig. 1. Measured above-ground and estimated below-ground C input of plant residues and animal manure in different treatments at all locations during 1997–2004 (a) and 2005–2008 (b), and at Foulum alone during 2005–2012 (c). In (a), (b) and (c), C inputs from above-ground plant residues and animal manure are shown above the reference line, and C inputs from below-ground plant residues are shown below the reference line.

comparing O2 and O4 at Foulum and Flakkebjerg (Table 4). Using catch crops enhanced SOC by 0.21 Mg C ha^{-1} yr^{-1}. The use of animal manure was not significant in enhancing SOC in the overall data analysis across all locations, but showed significant effects at both Jyndevad and Flakkebjerg (Table 4), where cattle slurry and anaerobically digested slurry was applied, respectively.

3.2.2. All sites, 2005–2008

During 2005–2008, there were little difference between treatments in SOC changes (Table 4). The cuttings of green manure was removed in +M treatments, and O2+CC-M became the treatment that best retained SOC in O2 at all sites (Table 4). In the analysis involving all locations, O2+CC-M was the treatment with the highest increase in SOC (Table 4). Generally, O4+CC+M was the treatment that lost most SOC. When the six fertilised treatments were compared, results showed that use of green manure in O2 did not significantly enhance SOC when compared to the rotation without green manure in O4 (Table 4). The organic rotation (O4) had significantly less SOC accumulation of 0.35 Mg C ha^{-1} yr^{-1} than the similar conventional (C4) rotation during the first four years of conventional farming (Table 4). Applying catch crops showed no significant effects at any site during this period.

3.2.3. Foulum, 2005–2012

From 2005–2012 at Foulum, O2+CC-M retained more SOC than other O2 treatments, likely because cuttings were retained in the −M treatment and removed in the +M treatments (Table 4). Overall,

Table 4
Mean change of soil organic carbon content (Mg C ha^{-1} yr^{-1}) in 0–25 cm depth in different treatments at Jyndevad, Foulum, Flakkebjerg during 1997–2004 (Jyndevad, Foulum and Flakkebjerg), 2005–2008 (Jyndevad, Foulum, Flakkebjerg) and 2005–2012 (Foulum). Analysis was also conducted with data of all three locations combined during 1997–2004 and 2005–2008. Part I of the table shows carbon inputs for individual treatment components and part II shows main effects of individual treatments. In part II treatments with –M were not used in analysis after 2004.

Treatment	Jyndevad		Foulum			Flakkebjerg		All locations	
	1997–2004	2005–2008	1997–2004	2005–2008	2005–2012	1997–2004	2005–2008	1997–2004	2005–2008
Part I									
O2-CC-M	0.10 ^{ab}	–	−0.84 ^{abc}	–	–	−0.18 ^{cd}	–	−0.30 ^{bc}	–
O2-CC + M	0.18 ^{ab}	−0.33 ^{ab}	−1.13 ^{bc}	−0.13 ^{a}	−0.17 ^{ab}	0.00 ^{abc}	−0.25 ^{abc}	−0.32 ^{bc}	−0.25 ^{ab}
O2 + CC-M	−0.31 ^{b}	0.05 ^{a}	−0.66 ^{ab}	0.08 ^{a}	−0.15 ^{ab}	0.15 ^{ab}	0.07 ^{a}	−0.27 ^{b}	0.06 ^{a}
O2 + CC + M	0.40 ^{a}	−0.95 ^{bc}	−0.44 ^{a}	−0.02 ^{a}	−0.56 ^{b}	0.27 ^{a}	−0.01 ^{ab}	0.07 ^{a}	−0.34 ^{ab}
O4-CC-M	–	–	−1.26 ^{bc}	–	–	−0.65 ^{e}	–	−0.80 ^{d}	–
O4-CC + M	–	−1.02 ^{bc}	−1.16 ^{bc}	0.39 ^{a}	−0.20 ^{ab}	−0.37 ^{de}	−0.36 ^{abc}	−0.61 ^{cd}	−0.33 ^{ab}
O4 + CC-M	–	−0.53 ^{abc}	−1.23 ^{bc}	0.24 ^{a}	−0.46 ^{b}	−0.09 ^{bcd}	−0.61 ^{c}	−0.51 ^{bcd}	−0.30 ^{ab}
O4 + CC + M	–	−1.40 ^{c}	−1.26 ^{c}	0.58 ^{a}	0.13 ^{a}	−0.03 ^{abc}	−0.50 ^{bc}	−0.49 ^{bcd}	−0.44 ^{b}
C4-CC + IF	–	−0.17 ^{ab}	–	0.11 ^{a}	−0.30 ^{ab}	–	0.19 ^{a}	–	0.04 ^{a}
C4 + CC + IF	–	−0.81 ^{abc}	–	0.64 ^{a}	−0.53 ^{b}	–	−0.16 ^{abc}	–	−0.11 ^{ab}
Part II									
O2	–	−0.64 ^{ab}	−0.77 ^{a}	-0.08 ^{b}	−0.37 ^{b}	0.06 ^{a}	−0.14 ^{ab}	−0.21 ^{a}	−0.30 ^{ab}
O4	–	−1.21 ^{b}	−1.23 ^{b}	0.48 ^{a}	−0.04 ^{a}	-0.28 ^{b}	−0.43 ^{b}	−0.61 ^{b}	−0.38 ^{b}
C4	–	−0.49 ^{a}	–	0.38 ^{ab}	−0.41 ^{b}	–	0.01 ^{a}	–	−0.03 ^{a}
-CC	0.14 ^{a}	−0.51 ^{a}	−1.10 ^{a}	0.12 ^{a}	−0.22 ^{a}	−0.30 ^{b}	−0.14 ^{a}	−0.51 ^{b}	−0.18 ^{a}
+CC	0.04 ^{a}	−1.05 ^{a}	−0.90 ^{a}	0.40 ^{a}	−0.32 ^{a}	0.07 ^{a}	−0.23 ^{a}	−0.30 ^{a}	−0.30 ^{a}
-M	−0.10 ^{b}	–	−1.00 ^{a}	–	–	−0.19 ^{b}	–	−0.48 ^{a}	–
+M	0.29 ^{a}	–	−1.00 ^{a}	–	–	−0.03 ^{a}	–	−0.34 ^{a}	–

For each group (treatments in Part I; rotations, catch crops and manure respectively in Part II) mean values having different letters within a column are significantly different at the 0.05 significance level.

O4 + CC + M had the highest SOC increase, while O2 + CC + M and C4 + CC + IF had the greatest decreases (Table 4). Considering only fertilised treatments in different rotations, O4 had significantly less SOC decrease than O2 and C4. During this eight-year period, using catch crops did not significantly affect SOC at Foulum (Table 4).

3.3. Effects of C input on SOC

When combining data from all sites in model I, around 82% ($p < 0.01$) of SOC in topsoil in 1997 was estimated to remain in 2008, with an estimated annual soil decomposition rate of 1.6% (Table 5). The estimated humification rate of C input from plant materials was about 12% ($p < 0.01$). For animal manure about 14% ($p > 0.05$) was estimated to be retained, but this value was associated with considerable uncertainty. Aboveground and belowground plant materials were estimated to contribute 4% ($p > 0.05$) and 19% ($p < 0.01$), respectively, of their C to SOC as shown in model II (Table 5). However, the standard error of the humification coefficients of above- and belowground plant material was about 6% in model II compared to 2% for total plant biomass in model I. Lower AIC and RMSE values of model I compared to model II also indicated that the use of separate humification coefficients for above- and below-ground plant material cannot be justified (Table 5). For individual sites, model I showed negative contribution from animal manure in some cases, while model II showed

Table 5
Estimated contribution of C input (Mg C ha^{-1}) from plant materials, animal manure (AM) and from original SOC content in top soil (0–25 cm in 1997) to the SOC content in 2008 at Jyndevad, Foulum and Flakkebjerg, and in 2012 at Foulum using two different models. Estimations were also performed with data of all three locations combined during 1997–2008. Values in brackets show the standard error.

		Jyndevad	Foulum	Foulum	Flakkebjerg	All locations
		1997–2008	1997–2008	1997–2012	1997–2008	1997–2008
	N	63	64	64	62	189
Model I	K_P (%)	17 (5.2) **	9 (5.7)	11 (4.2) **	17 (3.5) **	12 (2.3) **
	K_{AM} (%)	27 (22.8)	−10 (52.2)	40 (33.8)	−5 (24.6)	14 (18.7)
	K_O (%)	76 (5.8) **	85 (4.3) **	76 (4.1) **	73 (4.7) **	82 (2.2) **
	D_C (% yr^{-1})	2.2	1.4	1.7	2.6	1.6
	AIC	344.3	409.2	403.9	308.9	1076.9
	RMSE (Mg C ha^{-1})	3.29	5.34	5.03	2.64	3.93
	R^2	0.54	0.62	0.63	0.63	0.61
Model II	K_A (%)	35 (9.6) **	−12 (17.7)	13 (12.4)	−8 (8.3)	4 (6.3)
	K_B (%)	−1 (10.1)	35 (22.3)	8 (14.1)	43 (8.9) **	19 (6.5) **
	K_{AM} (%)	23 (22.2)	−1 (52.7)	41 (34.2)	9 (23.3)	16 (18.7)
	K_O (%)	77 (5.7) **	86 (4.2) **	76 (4.2) **	76 (4.5) **	83 (2.1) **
	D_C (% yr^{-1})	2.1	1.3	1.7	2.3	1.5
	AIC	341.4	407.8	404.8	301.1	1078.0
	RMSE (Mg C ha^{-1})	3.19	5.34	5.07	2.45	3.94
	R^2	0.57	0.62	0.62	0.68	0.61

Model I: $C_t = K_P \times C_{plant} + K_{AM} \times C_{AM} + K_O \times C_o$; Model II: $C_t = K_A \times C_{aboveground} + K_B \times C_{belowground} + K_{AM} \times C_{AM} + K_O \times C_o$; **: $0.0001 < P < 0.01$; AIC: Akaike information criterion; D_C: Decomposition rate of original SOC.

negative contributions of plant materials in some cases (Table 5). The large standard error for the humification coefficient for animal manure was probably an effect of the low input of C in manure compared with C input in plant material (Fig. 1).

During 16 years at Foulum (1997–2012), the estimated annual decomposition rate of original SOC was estimated as 1.7% (Table 5). The humification coefficient of plant materials was about 11% ($p < 0.01$) of C input. The humification coefficient for animal manure was considerably higher than for other analyses, about 40%, but with a standard error of 34% (Table 5). Splitting C input from plant materials as aboveground and belowground parts in model II showed similar contribution in both parts of around 10%, but none of these were statistically significant (Table 5). Model I had a lower AIC compared to model II, again indicating that a common humification coefficient for above- and belowground plant residues could be used.

4. Discussion

4.1. Effects of green manure, catch crops and animal manure on SOC change

4.1.1. Green manure

Including grassland in crop rotations is known to increase soil C (Leifeld, 2012), and generally the increase of SOC ranged from 0.3–1.9 Mg C ha^{-1} yr^{-1} (Christensen et al., 2009; Müller-Stöver et al., 2012; Rosenzweig et al., 2016). A previous monitoring study in Denmark also found significant increase of SOC by 0.9 Mg C ha^{-1} yr^{-1} in 0–25 cm soil layer for each year of grass in a crop rotation (Taghizadeh-Toosi et al., 2014). Our study showed that the inclusion of green manure significantly helped adding 0.4 Mg C ha^{-1} yr^{-1} more SOC in 0–25 cm soil depth (Table 4) during 1997–2004. Since the green manure only occupied 25% of the crop rotation, this corresponds to an additional 1.6 Mg C ha^{-1} yr^{-1} for each year of grass, which is slightly above the estimate of Taghizadeh-Toosi et al. (2014). Accordingly, using green manure increased total C input, even if it caused less C input from crops and catch crops (Fig. 1). The benefit of using green manure could be attributed to the large increment of C input, especially in C from root materials. The advantage in soil C retention by inclusion of green manure crops was also observed in O2 + CC-M compared to O4 + CC-M at all locations, even after 2004 (Table 4).

However, during 2005–2008 the advantage in SOC retention disappeared in O2 compared with O4, because grass-clover in the green manure was harvested and removed in +M treatments (Fig. 1), and the gap in C input between O2 and O4 was narrowed to less than 0.2 Mg C ha^{-1} yr^{-1} (Table 3). This also reduced the ability of O2 compared to O4 to retain SOC (Table 4). This indicates that an organic system with a harvested green manure crop does not necessarily retain more SOC than a system without green manure crops.

4.1.2. Catch crops

From 1997–2004, catch crops enhanced SOC on average by 0.2 Mg C ha^{-1} yr^{-1}, even though only significantly at Flakkebjerg (Table 4). However, using catch crops significantly increased estimated C input at all sites and most of this originated from C in catch crops (Fig. 1). After 2005, C input from catch crops decreased (Table 3), because of changes in catch crop management to enhance yield (Shah et al., 2017). However, an enhancing effect of catch crops on SOC was no longer observed at any of the sites (Table 4), even if there were still more C input in treatments with catch crops (Fig. 1). Considering the stability of more C input in treatments with catch crops (Table 3), the inconsistent SOC change may not be simply related to the quantity of C input from catch crops. In addition to higher C inputs, using rapidly decomposable material like catch crops could enlarge microbial community and enhance energy supply, accelerating the decomposition of more stable soil organic matter (Chen et al., 2014; Poeplau and Don, 2015), which would compromise the effect of enhanced C inputs from catch crops. Moreover, change in crop management and climate conditions could also cause uncertainties to the decomposition of catch crops (Kaspar et al., 2006; Steele et al., 2012). Also, high spatial variability of SOC makes it difficult to detect the small SOC changes after application of catch crops (Poeplau and Don, 2015).

4.1.3. Animal manure

From 1997–2004, application of animal manure did not significantly enhance SOC when all locations were considered, while at both Jyndevad and Flakkebjerg a significant effect was observed (Table 4). The average C input with animal manures at all sites was only about 0.2 Mg C ha^{-1} yr^{-1}. However, animal manure increased overall C input to 0.6 Mg C ha^{-1} yr^{-1} due to the higher input from crops (on average over 0.4 Mg C ha^{-1} yr^{-1}), resulting from higher crop productivity due to enhanced nutrient supply (Maillard and Angers, 2014). However, the expected increases in SOC were difficult to detect, which may also be related to the type of animal manure used (Maillard and Angers, 2014). In our study, cattle slurry (0.3 Mg C ha^{-1} yr^{-1}), pig slurry (0.2 Mg C ha^{-1} yr^{-1}) and anaerobically digested slurry (0.2 Mg C ha^{-1} yr^{-1}) were respectively used at Jyndevad, Foulum and Flakkebjerg during the first 8 years. Higher soil C content was observed in a large number of European long-term experiments with application of cattle manure (Zavattaro et al., 2017). In a review, Maillard and Angers (2014) found that animal manure from different animal species had different impacts, and that cattle manure led to significant positive SOC change, while pig and poultry manure did not. Domingo-Olivé et al. (2016) also observed that dairy cattle manure increased SOC significantly, while pig slurry did not. The different impacts between manures from cattle and pig were generally ascribed to the higher stability of organic matter in cattle manure (Velthof et al., 2000). Some reports also showed insignificant effects on SOC change after applying pig slurry (Rochette et al., 2000; Plaza et al., 2004).

4.2. SOC change under organic farming and conventional farming

The organic farming system O4 had higher C input compared with the similar conventional system (C4) during 2005–2008 at all three locations (Table 3), where C inputs from all plant residues were similar in both rotations, while a higher C input was estimated from root materials in O4 (Fig. 1b). In contrast to the estimated C inputs, SOC in O4 declined more than in C4 (Table 4). However, this was not the case at Foulum, where O4 accumulated more SOC than C4. This could indicate biases in the estimation of SOC inputs between the two treatments, and since aboveground C inputs were based on measurements, it is likely that the bias may have occurred with the estimation of belowground C inputs. Our estimates of root C inputs were based on measurements in the experiment in some of the years, but we did not have measurements in all years, and there were in general few measurements comparing catch crop roots between organic and conventional systems. This therefore points to the need for further studies to quantify root C inputs in the different cropping systems.

Treatments in conventional farming were converted from O2-CC-M and O4-CC-M, which received very limited nutrients during 1997–2004. Research have shown that unfertilised treatments have much lower microbial biomass and enzyme activities than treatments fertilised with pig slurry or cattle manure (Plaza et al., 2004; Francioli et al., 2016), while use of mineral fertilisers did not lead to significant increase in either microbial biomass or enzyme activities in a 4-year field experiment (Plaza et al., 2004). Moreover, Lori et al. (2017) reported that globally conventional farming had lower microbial abundance and activity than organic farming, based on meta-regression. Studies from the present long-term experiments showed significantly lower microbial biomass in the conventional treatments compared with the organic treatments at all sites during 2007 and 2008 (Petersen et al., 2013). These differences in microbial activity may have affected turnover of the added organic matter resulting in slower decomposition in conventional versus organic treatments, which may also have contributed to differences in SOC between O4 and C4.

Considering the C input from 2005 to 2012 at Foulum, straw of cereals in C4 was removed from 2010, thus enlarging the advantage of O4 in C input quantity over C4 (Table 3). In addition, there was a higher C input from plant materials in O4, especially from roots (Fig. 1c). In contrast to results for 2005–2008, SOC change during 2005–2012 at Foulum showed that O4 retained more SOC than C4 (Table 4). This agrees with the estimated higher C input in O4 compared with C4. A possible reason could be that over time the use of mineral fertilisers have increased microbial activity of the conventional farming systems thus giving similar turnover rates in O4 and C4, resulting in SOC changes reflecting C inputs. Also, removing straw of cereals reduced C input after 2010, which may have affected measured SOC in C4 in 2012.

4.3. Contribution of C input from aboveground and belowground biomass to SOC

Belowground C has been found to be better retained in soils than C from aboveground plant parts (Rasse et al., 2005; Kätterer et al., 2011; Berti et al., 2016). However, this was not indicated from our analyses of relations between C inputs and SOC changes. After 2005, the significant ability of O2 in enhancing SOC over O4 disappeared, and at the same time, a practice of removing the aboveground part of green manure in O2 started (Table 4). The estimation of root C input still indicated higher inputs in O2 compared with O4 (Fig. 1b). Moreover, removing cereal straw in C4 after 2010 seemed to enhance the difference in SOC change between C4 and O4 at Foulum (Table 4). These results indicate that aboveground C input also contributed considerably to topsoil SOC. Similarly, in the regression analyses between SOC change and C inputs (Table 5), models that used whole plant material as input performed better than models where C input were divided between above- and belowground parts. Austin et al. (2017) observed in their reciprocal litter transfer experiment that aboveground plant material was more decomposed than belowground materials after 5 months, while this difference disappeared after 17 months. This may be related to the chemical properties of above- versus belowground materials (Rasse et al., 2005), which in particular plays a role during the initial phase of organic matter decomposition, but may be less important over a longer time span (Gentile et al., 2011). Our statistical analyses covered periods of more than 10 years, which may have hidden the short-term effects and reduced the differences in degradability of root versus aboveground materials. Also, we studied arable systems with regular tillage, which may stimulate degradation of below- and aboveground residues to similar extents.

5. Perspectives

Using green manure, catch crops and animal manure each increased C input of 0.6–1.0 $Mg\,C\,ha^{-1}\,yr^{-1}$ at crop rotation level. However, only the effect of green manure was consistently reflected in observed SOC change. This calls for more detailed studies on measurements of C inputs from various sources and how the C inputs are degraded and contributes to stabilized SOC. In particular, better estimation of C inputs from roots and root exudates is highly needed to assess the effect of cropping systems and crop management on SOC. We applied a fixed amount of C inputs in roots, whereas other approaches estimate root C input to be proportional to aboveground biomass. The latter approach would have given different estimated belowground C inputs between the systems. This illustrates the needs to develop and consolidate methods for improved estimation of belowground C input as also found in a recent model-based comparison (Keel et al., 2017).

Including grass crops in the crop rotation has been widely reported to increase soil C (Leifeld, 2012; Taghizadeh-Toosi and Olesen, 2016). However, grassland management also has considerable effects on SOC (Conant et al., 2017) and this should be considered when evaluating the effects of organic farming systems on total greenhouse gas emissions. This is illustrated by the effect in our study of removing grass-clover cuttings as compared to mulching these in the field. This removal of grass-clover in the experiment was meant to simulate a situation where the grass-clover was used for biogas and the digested slurry was used to fertilise the other crops in the rotation. This approach enhanced yields of the arable crops in the rotation by about 30% (Shah et al., 2017), which also enhanced C inputs from crop residues (Fig. 1b). However, this could not make up for the C not returned in mulched grass-clover. It is therefore challenging agronomically in organic arable systems to achieve both high yields and high soil C inputs.

Our study showed slightly higher estimated C inputs in conventional compared with similar organic cropping systems (Table 3), whereas measured SOC changes showed opposite trends although this was reversed over time at Foulum (Table 4). This contrasts with other studies that showed consistently higher SOC accumulation in organic compared with conventional systems (Gattinger et al., 2012). In reality, there may be considerable variation between different organic and conventional systems in their ability to enhance SOC, and factors such as use of green manure, catch crops and residue management play a large role.

6. Conclusions

Including a whole-year green manure with mulching of crop residues in organic cropping systems increased C input with about 0.9 $Mg\,C\,ha^{-1}\,yr^{-1}$, and enhanced SOC by 0.4 $Mg\,C\,ha^{-1}\,yr^{-1}$ as average over a 4-year rotation. However, this effect largely disappeared when cuttings of the green manure crop was removed. Catch crops were estimated to contribute nearly 1.0 $Mg\,C\,ha^{-1}\,yr^{-1}$ of additional C input, whereas the effect on SOC could not broadly and consistently be observed. Similarly, the advantage of applying animal manure on SOC was not observed at all locations, even though animal manure across all locations increased the C input with about 0.7 $Mg\,C\,ha^{-1}\,yr^{-1}$, where over 0.4 $Mg\,C\,ha^{-1}\,yr^{-1}$ of the extra C input was due to increased crop production and more crop residues. Compared with conventional farming, organic farming systems had more total C inputs, in particular from estimated belowground plant materials and added animal manure. Analysis of the relation between changes in SOC and inputs of C in above- and belowground plant residues and animal manure could not differentiate effects of above- and belowground C input, and these inputs may therefore be considered as having similar effects on SOC.

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Paper III

Release of C and N from fodder radish (*Raphanus sativus*) shoots and roots incubated in soils with different management history

Short title: **Radish shoot and root decomposition**

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Abbreviations: CC, cover crop; IF, inorganic fertilizer; M, manure

Core ideas:

- Shoot-C mineralized faster than root-C in the initial phase of decomposition
- The longer-term release of C did not differ between shoots and roots
- Greater mineralization of N from shoots than from roots
- Soil management history had only little impact on C and N mineralisation
- Radish root-N preserved better against mineralisation than N in shoots

Abstract

It is generally acknowledged that plant roots decompose more slowly than shoots and contribute more to accumulation of soil organic matter, and that management history shapes the structure and function of decomposer communities in soil. Here we study the effect of chemical characteristics on the release of carbon (C) and nitrogen (N) in shoots and roots from fodder radish (*Raphanus sativus oleiformis* L.), a widely used nitrate catch crop. Shoots and roots were incubated separately for 180 days at 20 °C using four soils with different management history, and the release of CO_2 and extractable mineral N was determined. Shoot C mineralized faster than C in roots during the first weeks of incubation. After 180 days, 58% of the C input was mineralised with no difference between shoots and roots. Shoots had a narrower C/N ratio than roots, and at the end of incubation, shoots had released more N (42% of shoot N) than roots (28% of root N). Soil with a management history including frequent use of catch crops and animal manure had higher initial content of mineral N and released more C during incubation, but management history did not affect release of applied plant C. Residues incubated in soil with a management history involving catch crops showed slightly higher N mineralisation. Longer-term decomposition of C added in radish shoots and roots is unaffected by differences in chemical characteristics and soil management history, while the net mineralization of N in shoots is faster than for N in roots.

Introduction

In temperate soils with net percolation during the autumn and winter periods, nitrate catch crops are common management elements in organic as well as conventional farming. Catch crops retain nitrate that would otherwise be lost from the soil by leaching and long-term use of catch crops leads to higher soil C and N content (Thomsen and Christensen, 2004; Constantin et al., 2010). Fodder radish (*Raphanus sativus oleiformis* L.) has become a widespread nitrate catch crop and when left until spring, the crop is efficient in reducing nitrate leaching (Constantin et al., 2010; Sapkota et al., 2012; Thomsen and Hansen, 2014). However, the short-term N mineralization from fodder radish shoots is significant even at low temperatures (Thomsen et al., 2016), but information regarding the lability of C and N retained in radish roots is lacking.

It is widely reported that roots contribute more to soil organic matter build-up than biomass added in shoots (e.g. Kong and Six, 2010; Kätterer et al., 2011; Berti et al., 2016; Austin et al., 2017). The difference between shoots and roots ascribes partly to their different chemical properties. Compared with shoots, roots are most often lower in N content and contain higher concentrations of organic compounds (e.g. lignin, suberin) considered to retard decomposition (Rasse et al. 2005). However, there are indications that differences in the mineralization rate of roots and shoots relate only to early decomposition phases (Helfrich et al., 2008; Gentile et al., 2011).

Crop management may shape the structure of the soil microbial community leading to differences in their capacity to decompose various substrates (Yin et al., 2010; Mbuthia et al., 2015; Francioli et al., 2016). Incorporation of crop residues and application of animal manure provide nutrients and labile energy-rich organic substrates and create a more benign microenvironment for the soil microbial community, including increased moisture retention and gas exchange through improved soil structural stability and porosity (Jensen et al., 2017). However, the impact of soil management history on the decomposition of shoot and root residues from radish cover crops remains unclear.

In this study, we examine the release of C and N from shoot and root biomass of fodder radish incubated separately for 180 days in soils from a long-term field experiment. The soils had been under four different arable crop managements involving catch crops, use of animal manure or mineral fertilizers. The objectives were to establish any differences in the C and N release from shoot and root biomass and the impact of soil management history.

Materials and methods

Soils and management systems

In autumn 2012, we retrieved soil from the plough layer (0-25 cm) of a long-term field experiment located at Foulumgaard Experimental Station, Aarhus University, Denmark (56°30′N, 9°34′E). The soil is a loamy sand and classifies as Mollic Luvisol (FAO) and Typic Hapludult (USDA Soil Taxonomy) with 9 % clay (< 2 μm), 13 % silt (2 – 20 μm), 47 % fine sand (20 – 200 μm), 27 % coarse sand (200 – 2000 μm) and 4 % organic matter in the 0-20 cm soil (Sapkota et al., 2012). Average annual temperature and precipitation is 7.3 °C and 704 mm, respectively. The experiment has since 1997 encompassed different management systems; Table 1 shows the history of the four management systems sampled in this study. The management histories involve combinations of nitrate catch crops (±CC), and use of animal manure (+M; since 1997) or inorganic fertilisers (+IF; unfertilised 1997-2005). For each management system, we collected soil from eight replicate plots. Olesen et al. (2000) and Shah et al. (2017) provide further details on the field experiment.

Shoot and root materials for incubation

Shoots and roots of a fodder radish catch crop (*Raphanus sativus oleiformis* L.), sown on 4 August 2014 following harvest of a potato crop, were collected during 15 – 19 December 2014. The catch crop was established in rows 24 cm apart, and shoots and roots were sampled within three rectangular metal frames (35 cm ×24 cm) inserted 20 cm into the soil and covering one row of radish. Shoots were cut at ground level and oven-dried at 60°C for 42 h.

The soil enclosed by the metal frame was removed and kept at 2 °C until isolation of roots. The soil from each frame was sieved through a 1 cm mesh and large roots retained on the sieve collected. Soil passing the 1 cm mesh was mixed into 350-450 g portions; these were washed individually through a 0.425 mm sieve. Further removal of soil mineral particles from roots was by flotation and washing with tap water. Subsequently, root fractions with any particulate organic debris were transferred to a white tray and roots isolated based on colour and physical appearance. The root samples were oven-dried at 60°C for 42 h.

To eliminate any effect of differences in the physical/structural properties of shoots and roots, plant materials used for analyses and incubation were ball-milled.

Incubation and analyses

The soil for the incubation experiment, sampled 10 September 2015 following harvest of a spring wheat crop, was air-dried and sieved (< 2 mm) to remove plant residues and stones.

After mixing, sub-samples of the air-dry soil (140 g) were packed into steel cylinders (100 cm^3) using a manual tamper to reach a bulk density of 1.38 g cm^{-3}. In total 36 soil cylinders were prepared for incubation. After adding water to reach a matric potential of pF 2.0 (corresponding to a gravimetric soil water content of 16 %), the soil cylinders were pre-incubated in the dark at 20°C for one week.

After pre-incubation, the soil core was carefully pushed half-way out of the cylinder and cut into two equal halves. Radish shoot or root material (475 - 500 mg DM) was added to the exposed soil surface and the two halves of the soil core re-installed in the cylinder. In this way, plant materials became sandwiched in the middle of the soil core during incubation.

For each soil management history, the incubation included three replicates with shoots, three with roots, and three reference cylinders treated similarly but without radish residues. After weighing, each soil cylinder was placed in a 0.5 L respirometer jar. The jars were transferred to a temperature regulated RESPICOND VI respirometer (A. Nordgren Innovations AB) kept at 20°C. Each respirometer jar included a beaker with 20 mL 0.6 M KOH solution to trap the CO_2 evolved during incubation. Platinum electrodes measured the decrease in conductance of the KOH solution automatically over a period of 180 days; initial logging intervals were 30 min. and subsequently one hour. When the cumulated CO_2 reached about 80 mg in a jar, the KOH solution was replaced by a fresh solution. The release of CO_2-C from shoots and roots was estimated as the difference between CO_2 evolved from amended cylinders and reference cylinders without any amendments.

At the end of the incubation, the soil was removed from the cylinders and mixed. Then 25 g soil was extracted with 100 ml 1 M KCl in 250 ml polyethylene bottles that were shaken end over end for 1 hour. The extract was filtered through glass filters (GF/A) and kept at -20° until analysis. Ammonium and nitrate in the extract was subsequently determined on a Bran+Lübbe Autoanalyzer3 (Seal Analytical GmbH). The remaining soil was used for dry matter determination (105°C for 24h).

Total C and total N concentrations in air-dry and sieved (< 2 mm) soil were analysed by dry combustion using a Thermo Flash 2000 NC Soil Analyser (Thermo Fisher Scientific) for soils sampled in 2012 and a Vario MAX cube (Elementar Analysensysteme AG) for soil sampled in 2015. Plant materials were analysed for total C and N concentrations using dry combustion and a PDZ Europa ANCA-GSL elemental analyser (Sercon Ltd.). The plant samples were also analysed for content of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (Van Soest et al., 1991) using a Foss Fibertec 2010 System (Foss Analytical AB). Soil pH was measured by mixing 10 g air-dried soil with 25 ml water.

Data analysis

The General Linear Model (GLM) of SAS software was used for statistical analyses (SAS Institute 2008). The significance ($P<0.05$) of treatments was compared by calculating Least Significant Difference (LSD).

Results

Soil with a history of mineral fertilizers only and no catch crops (-CC+IF) was significantly lower in total-N than soil retrieved from alternative systems (Table 2). This was also true for soil C content, although the difference between –CC+IF and +CC+M did not appear as significant. Soils sampled in the systems +CC+M, -CC+M, and +CC+IF did not differ significantly in C and total-N concentrations. The concentration of mineral-N, initially present in soils used for incubation, was slightly smaller in soils with no history of catch crops, regardless of fertilization regime (Table 3). Soil with a history of animal manure and catch crops (+CC+M) showed somewhat higher mineral-N content. Of the mineral-N, nitrate accounted for 17.2 to 22.6 mg N kg^{-1} soil while the ammonium content was 0.9 to 1.1 mg N kg^{-1} soil (data not shown). The pH of soils from the four management histories differed little (Table 3).

Radish shoots had higher N concentrations than roots (Table 4). With similar C concentrations in these two plant parts, this translates into a smaller C/N ratio in shoots (12.5) than in roots (22.2). Roots also differed from shoots in terms of chemical composition; roots were higher than shoots in concentrations of neutral detergent fibre (NDF), acid detergent fibres (ADF) and in acid detergent lignin (ADL, also denoted lignin).

During the first 10 days, average net CO_2 release was significantly higher for shoots than for roots (Tables 5 and 6). During this period, 43% of the shoot C and 32% of the root C evolved as CO_2. Within the next 80 days, this pattern reversed; now significantly more C evolved from roots than from shoots. This closed the gap in cumulated CO_2 emissions between roots and shoots established during the first 10 days of incubation (Fig. 1). During the last 90 days of the incubation period, the release of CO_2 from incubated shoots and roots did not differ. After 180 days of incubation, the net release of C from roots and shoots averaged 58% with no significant difference between shoots and roots. In contrast, mineralization of N from shoots and roots differed significantly (Tables 5 and 6). During the 180 days of incubation, 42 % of the N added in shoots mineralized while roots only released 28 % of the N added initially.

For soils with a history of catch crops, the amount of CO_2 evolved during incubation from reference cylinders was higher for soil dressed with manure than for soils receiving mineral fertilizer (Table 3). Soil management history did not influence the release of CO_2 from roots during any period of the incubation (Table 5 and 6). For shoots, the release of CO_2 during the first 10 days was unaffected by soil management history. However, the release of CO_2 during day 11 to day 90 was smaller for shoots incubated in soil with a history of catch crops and manure (+CC+M) than for shoots incubated in soil with a history of catch crops and mineral fertilizers (+CC+IF). For other incubation periods, the release of shoot C did not differ between soil histories. The proportion of shoot N mineralized did not differ between soils. For N added with roots, the fraction mineralized in soil with a history of manure and no catch crops was somewhat smaller than the fraction of N mineralized from roots incubated in soil with alternative management histories (Table 5).

The release of N from added materials tended to be higher for treatments with a history of catch crops, although differences were not statistically significant for individual treatment combinations (Table 5). The average release of plant N was 37% in soils with a prehistory of catch crops and 33% in soils without a history of catch crops. The overall N mineralization was lower for roots incubated in soil with a history of manure and no catch crops (-CC+M; Fig. 2). Soils with no history of catch crop and incubated with shoots were lower in mineral N than soil with a history of catch crops and animal manure additions (+CC+M). Thus, there was a significant interaction between management history and the content of mineral-N in soils after incubation (Table 6), but for reference soils without amendments of shoot and root materials, soil management history had no effect on net N mineralisation.

Discussion

It is widely accepted that decomposing plant roots contribute more to refractory soil organic matter than aboveground biomass. However, much evidence for roots being decomposed more slowly than shoots relies on field studies with root biomass estimated indirectly by allometric functions (e.g. Kätterer et al., 2011; Berti et al., 2016) or studies based on pulse labelling with isotopes and subsequent litter-transfers with roots decomposing in-situ (e.g. Kong and Six, 2010; Austin et al., 2017). These experimental approaches are less feasible when attempting to relate the chemical composition of shoot and roots to their decomposition rates (Rasse et al., 2005). The use of allometric functions provides only crude estimates of root inputs (Taghizadeh-Toosi et al., 2016; Keel et al., 2017), and root decomposition in soil may vary considerably among different root size-classes and roots from different plant species (de Graaff et al., 2013; Redin et al., 2014).

In field studies, shoot residues incorporated by tillage and roots left undisturbed in-situ facilitate different conditions for microbial activity whereby environmental effects and differences in soil disturbance become confounded with effects related to the chemical composition of the plant residues. In their review, Rasse et al. (2005) ascribed only one-fourth of the difference in mean residence time in soils of root-derived versus shoot-derived C to differences in the chemical composition.

In the initial phase of decomposition, we observed a faster release of shoot C compared with C added in roots. The higher content of crude fibres and lignin may retard microbial attack on roots in this phase (Rasse et al. 2005). However, the subsequent rate of C release from roots surpassed that of shoots and at the end of the incubation period, the mineralized proportion of root and shoot C was similar. Thus, the impact of the different chemical characteristic of shoots and roots was short-term and compensated for in subsequent decomposition phases. This pattern is in accordance with results obtained by Helfrich et al. (2008) for maize leaves and roots incubated at 15°C for 84 days. Although the initial release of CO_2 was faster for maize leaves than for roots, this difference disappeared later and they concluded that roots did not contribute more than shoots to stabilized soil C. Gentile et al. (2011) concluded that while litter quality controlled the shorter-term C dynamics, initial litter characteristics do not predict longer-term retention of C in soil. Similarly, Abiven et al. (2005) found that root decomposition was not systematically lower than that of aerial plant parts and questioned the relationship between chemical characteristics of plant residues and their mineralisation rate in soil. On the other hand, Redin et al. (2014) found the chemical composition of roots, originating from 20 different crop plants and incubated with soil for 120 days at 25°C, to predict their release of C correctly.

In contrast to C, we found a distinct difference in the mineralization of N added in radish shoots and roots. During the 180 days of incubation, shoots mineralized considerably more N than roots. The release of N from shoots corresponded to 42 % of the N added initially. A previous study involving aboveground radish residues of different age showed that 40 to 50 % the residue N mineralized when incubated at 2 and 10 °C for 7 month (Thomsen et al., 2016). The release of N added with roots was considerably smaller; only 28 % was mineralized during the incubation period. We ascribe the greater N release from shoots to a higher N concentration and narrower C/N ratio. Employing a wide range of plant materials incubated for 30 weeks at 15 °C, Jensen et al. (2005) found a close correlation between initial plant N concentration and N mineralisation. The correlation between mineralisation of N and plant C/N- or lignin/N-ratio was poorer.

Comprehensive studies of microbial properties of soils maintained under different management indicate that crop rotational diversity, fertilization regime, tillage system, soil pH, and use of cover crops affect the structure and composition of the microbial community (Yin et al., 2010; Mbuthia et al., 2015; Francioli et al., 2016). The impact of management history include different abundance of microbial groups with specific functional potentials. However, the implications of changes in microbial biodiversity for mineralisation of C and N added with crop residues remains unclear. The vast majority of microorganisms in soil show wide niche overlaps when it comes to generic processes such as crop residue decomposition.

In our study, cropping sequences adopted in the four management systems were almost identical (Table 1) and tillage operations did not differ. The main differences were the amount and type of nutrient source (manure or mineral fertilizer) and the use of cover crops (N_2-fixing crops in systems with animal manure and non-fixing cover crops in systems with mineral fertilizers). The N input from N_2-fixing cover crops was partly compensated for by reduced nutrient inputs in animal manure. Despite their different long-term inputs of residues from various catch crops and different nutrient sources, the four soils differed little in pH and in concentrations of C and N. Apparently, the choice of main crop sequence and tillage system was the defining factors for the soil organic matter level in this sandy soil with moderate fertilization. However, the soil with a history of mineral fertilizer only (and no N from 1997 to 2004) and no catch crops showed reduced levels of soil C and total N.

The long-term management history did not affect the release of C from radish shoots and roots, suggesting that the different management histories had little effect on the performance of the microbial communities. However, more soil N mineralised when plant material was added to soils with a history of catch crops (+CC) compared to soils without catch crops (-CC). This may indicate a reduced N immobilization potential in soil with a history of catch crops. For soil with a history of catch crops and animal manure, the initial content of mineral N was also higher. However, the amount of soil N mineralised during incubation was similar to that of soils with alternative management histories.

Conclusions

This incubation study examined the effect of chemical characteristics on the release of C and N added with shoots and roots from fodder radish. While the release of C was similar for shoots and roots, shoots mineralized a larger fraction of the added N than did roots. The observed differences relate only to chemical characteristics, which according to Rasse et al. (2005) may account for only one-fourth of the retention of C that can be obtained for roots in-situ. Assuming that N in aboveground plant biomass will be more prone to mineralisation when terminated in the autumn or in early winter by frosts, we expect that N retained in catch crop roots left in-situ over-winter is better protected against mineralization and subsequent loss by leaching than N accumulated in the aboveground radish biomass.

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Tables and figures

Table 1. Management history of the four soils used in the present incubation study. Management histories with inorganic fertilizers (+IF) were kept unfertilized and without a catch crop from 1997 to 2005.

Management history	-CC+M		+CC+M			-CC+IF		+CC+IF		
	Crop sequence	M[†]	Crop sequence	M[†]	CC[‡]	Crop sequence	IF[†]	Crop sequence	IF[†]	CC[‡]
1st cycle	S. oats	40	S. oats	40	+[#]	S. oats	0	S. barley:ley	0	
1997-2000	W. wheat	70	W. wheat	70	+[#]	W. wheat	0	Grass-clover	0	
	W. cereal	70	W. cereal	70	+[#]	W. cereal	0	W. wheat	0	
	Pea/barley	0	Pea/barley	0	+[¶]	Pea/barley	0	Pea/barley	0	
2nd cycle	W. wheat	50	W. wheat	50	+[¶]	W. wheat	0	S. barley:ley	0	
2001-2004	S. oats	50	S. oats	50	+[¶]	S. oats	0	Grass-clover	0	
	S. barley	50	S. barley	50	+[§]	S. barley	0	W. cereal	0	
	Lupin	0	Lupin	0		Lupin	0	Lupin	0	
3rd cycle	S. barley	60	S. barley	60	+[¶]	S. barley	130	S. barley	130	+[§]
2005-2009	F. beans	0	F. beans	0	+[¶]	F. beans	0	F. beans	0	+[§]
	Potato	110	Potato	110		Potato	140	Potato	140	
	W. wheat	110	W. wheat	110	+[¶]	W. wheat	165	W. wheat	165	+[§]
4th cycle	S. barley	60	S. barley	60	+[¶]	S. barley	120	S. barley	120	+[§]
2010-2014	Hemp	90	Hemp	90		Hemp	125	Hemp	125	
	Peas/barley	0	Peas/barley	0	+[¶]	Peas/barle y	0	Peas/barley	0	+[§]
	S. wheat	100	S. wheat	100	+[¶]	S. wheat	110	S. wheat	110	+[§]
	Potato	100	Potato	100	+[¶]	Potato	140	Potato	140	+[§]

† M: Manure application rates in +M treatments (kg NH_4-N ha^{-1} in 1st and 2nd cycles and kg total-N ha^{-1} in 3rd and 4th cycle). IF: Inorganic fertilizer rates (kg N ha^{-1}).

‡ CC: Catch crops in +CC treatments.

§ Monocultures or mixtures of non-N_2-fixing catch crop.

¶ Mixtures of N_2-fixing and non-N_2-fixing catch crop.

White clover.

Table 2 Total C and N concentrations in soil (0-25 cm) sampled in November 2012 for determination of impact of management history on soil organic matter content. Soil was sampled from 8 plots per treatment.

Management history	Total C ($g\ C\ kg^{-1}$ soil)	Total N ($g\ N\ kg^{-1}$ soil)
+CC+M	19.5 ± 3.4 ^{ab} †	1.70 ± 0.13 ^{a}
-CC+M	21.3 ± 1.8 ^{a}	1.70 ± 0.07 ^{a}
+CC+IF	20.5 ± 2.8 ^{a}	1.73 ± 0.18 ^{a}
-CC+IF	17.9 ± 4.1 ^{b}	1.51 ± 0.22 ^{b}

† Mean ± S.D. (n=8), mean values with different letters in the same column are significantly different at the 0.05 significance level.

Table 3 Characteristics of soil (0-25 cm) sampled in September 2015 for the incubation study and the amount of C evolved as CO_2 from the reference soils during the 180 days of incubation.

Management history	pH (H_2O)	Total N ($g N kg^{-1}$ soil)	Total C ($g C kg^{-1}$ soil)	Mineral N at day 0 ($mg N kg^{-1}$ soil)	Cumulated CO_2 evolved in 180 days (reference soils) ($mg CO_2$-$C g^{-1}$ soil)
+CC+M	6.3	1.73	20.8	23.6 ± 0.1 [a] †	0.48 ± 0.04 [a]
-CC+M	6.4	1.67	21.2	18.8 ± 0.0 [c]	0.44 ± 0.03 [ab]
+CC+IF	5.9	1.73	21.8	20.1 ± 1.3 [b]	0.37 ± 0.03 [b]
-CC+IF	6.0	1.56	19.4	18.3 ± 0.5 [c]	0.43 ± 0.07 [ab]

† Mean ± S.D. (n=3), mean values with different letters are significantly different at the 0.05 significance level.

Table 4 Characteristics of plant materials used for incubation and the amount of C and N added to each incubation cylinder in shoot or root material. NDF and ADF is neutral detergent fibre and acid detergent fibre, respectively.

Plant part	C	N	NDF	ADF	Lignin	Total C	Total N	C/N
	mg g^{-1} dry matter					mg incorporated per cylinder		
Shoot	385	30.8	345	326	28	182	15	12.5
Root	395	17.8	599	507	70	192	9	22.2

Table 5 Net CO_2-C evolved and net N mineralised during incubation from soils incubated with shoot or root materials in soils with different management histories (C and N mineralised from reference soils subtracted). LSD based on n=3.

Plant part	Management histories	Net CO_2-C evolved (% of applied C)				Net N mineralised (% of applied N)
		0-10 Days	11-90 Days	91-180 Days	0-180 Days	0-180 Days
Shoot	+CC+M	43.8 [a] †	11.3 [c]	1.3	56.3	43.2 [a]
	-CC+M	44.0 [a]	13.7 [bc]	1.3	58.9	40.7 [a]
	+CC+IF	42.3 [a]	18.4 [ab]	3.7	64.4	43.4 [a]
	-CC+IF	42.1 [a]	13.2 [bc]	1.5	56.9	39.8 [a]
Root	+CC+M	31.8 [b]	20.7 [a]	3.2	55.7	29.0 [b]
	-CC+M	32.2 [b]	22.6 [a]	2.7	57.6	22.1 [c]
	+CC+IF	31.5 [b]	24.7 [a]	2.7	58.9	31.4 [b]
	-CC+IF	31.3 [b]	18.6 [ab]	1.8	51.6	28.0 [b]
LSD (P<0.05)		2.5	6.8	NS	NS	5.8

† Mean values with different letters within a column are significantly different at the 0.05 significance level.

Table 6 Statistical test of the effect of plant part (shoots, roots) and management history on net release of C and N during incubation. †

	Net CO_2-C evolved				Net N mineralised
	0-10 Days	11-90 Days	91-180 Days	0-180 Days	0-180 Days
R^2	0.959	0.663	0.155	0.272	0.884
Plant part	***	**	NS	NS	***
Management history	NS	NS	NS	NS	*
Plant part × Management history	NS	NS	NS	NS	NS

† ***, P < 0.0001; **, 0.0001 < P < 0.01; *,0.01<P < 0.05; NS, P > 0.05.

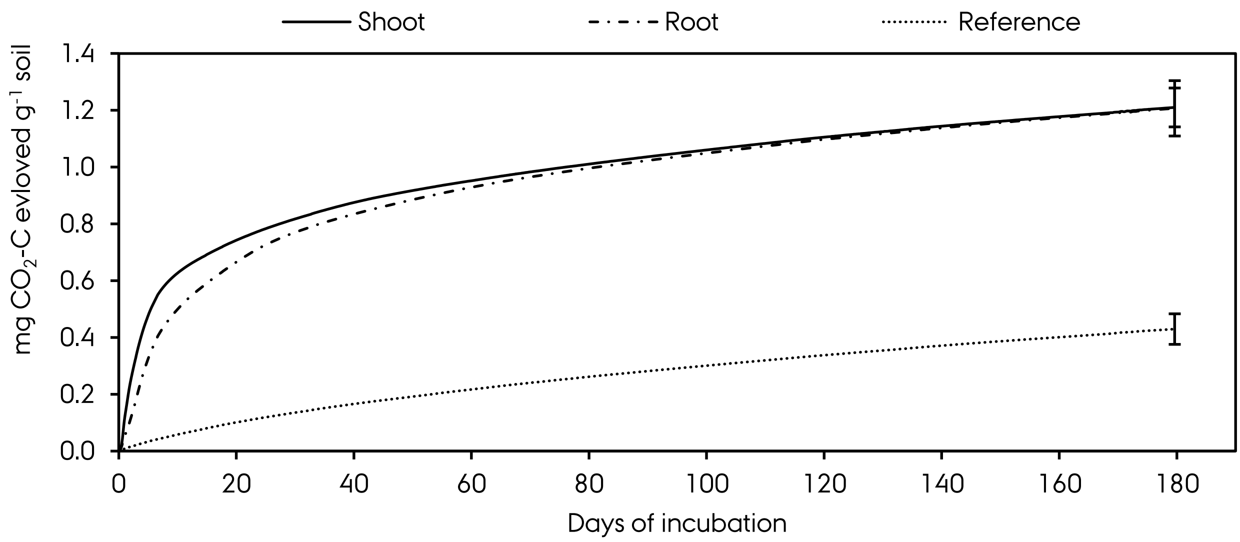

Fig. 1 Cumulated CO_2-C evolution from reference soil with no amendment and soil amended with shoots or roots during the incubation period (180 days). Average of four soils with different management history. Bars indicate standard deviation (n=12).

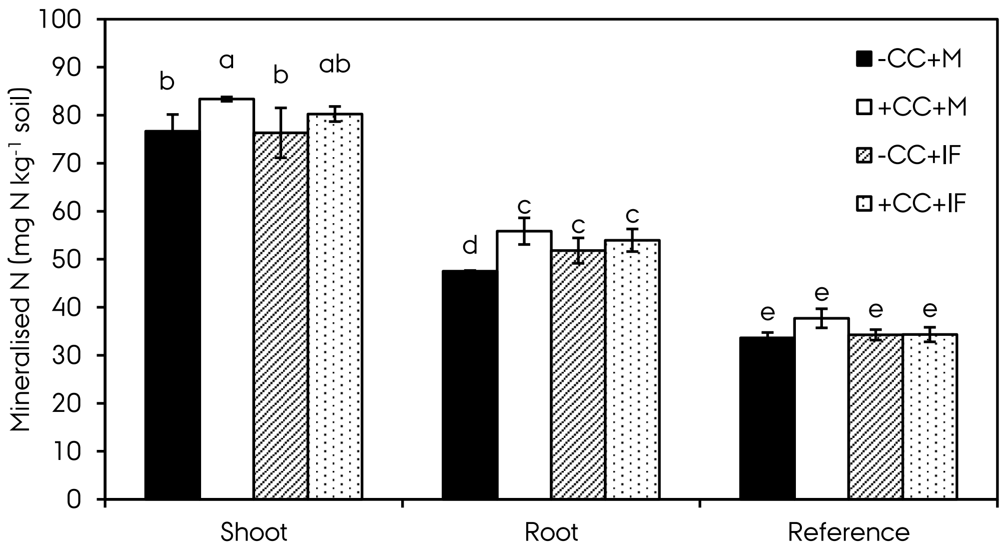

Fig. 2 Nitrogen mineralisation in reference soil with no amendment and soil amended with shoots or roots measured after the 180 days incubation period (mineral N present in soil at day 0 subtracted). The soil had four different management histories (see Table 1 for legend). Bars indicate standard deviation (n=3).

Paper IV

Converting temperate long-term arable soil into semi-natural grassland: decadal-scale changes in topsoil C, N, ^{13}C and ^{15}N contents

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Abstract

Converting arable soil into permanent grassland remains an efficient management option for increased C storage in agricultural land. In this study, we quantify changes in C and N in topsoil from the Sandmarken fertilizer experiment (initiated 1894 at Askov Experimental Station, Denmark) before and after its conversion in 1998 into unfertilized semi-natural grassland. Due to different fertilization in the arable phase (unmanured (0), animal manure (AM) and mineral fertilizer (NPK)), the grass grew on soil plots with different initial fertility. Archived soils sampled during 1942-2012 from plots subject to 0, NPK and AM treatment in the arable phase were analysed for C, N, ^{13}C and ^{15}N. Using crop yields (1923-1997), topsoil C contents (1923-2012) and the C-TOOL model, we estimated C inputs in the arable and grassland phase. For the arable phase, allometric functions showed a mean annual C input of 0.4, 1.4 and 1.7 Mg C ha^{-1} for 0, NPK and AM plots, respectively. In the grassland phase, modelled input of C ranged 3.2-3.8 Mg C ha^{-1}. In the arable phase, topsoil showed mean annual losses of 0.10 Mg C ha^{-1} and 0.012 Mg N ha^{-1}, while $\delta^{13}C$ increased by 0.0021 ‰ and $\delta^{15}N$ by 0.014 ‰. Grassland establishment reverted losses of C and N to annual gains of 0.29 Mg C ha^{-1} and 0.017 Mg N ha^{-1}; $\delta^{13}C$ now decreased by 0.063 ‰ and $\delta^{15}N$ by 0.074 ‰. Fertilization history did not affect these changes significantly, although soil C and N levels remain different in both the arable and grassland phase (0 < NPK < AM). Converting this low-yielding sand soil from arable into semi-natural grassland provided an overall annual gain of 0.39 Mg C ha^{-1} and 0.029 Mg N ha^{-1} in the topsoil. Changes in $\delta^{13}C$ and $\delta^{15}N$ indicated a reduced C turnover rate and a less leaky N cycle under grassland.

Keywords: Land use change, topsoil C and N, Sandmarken long-term experiment, arable rotation, semi-natural grassland.

Introduction

Numerous studies document that changes in land use can have substantial impacts on the amount of C stored in soil (e.g. Post and Kwon, 2000, Guo and Gifford, 2002; Freibauer et al., 2004; Poeplau et al., 2011; Kämpf et al., 2016; Kopittke et al., 2017). Most studies show that the largest losses occur when soils under native vegetation turn into soils under arable rotation. In temperate climates, long-term arable management may reduce the pre-arable soil C content by 30 to 60 % (Gou and Gifford, 2002; Poeplau et al., 2011; Kopittke et al., 2017). In particular, large losses occur when converting permanent grasslands into arable rotations dominated by annual crops (Johnston et al., 2009; Gregory et al., 2016) with losses of C occurring rapidly in the early phase after conversion and then slowing down as the arable use continues.

The 4-‰ research initiative, launched at the COP-21 conference in Paris (Minasny et al., 2017) as a means to combat increasing atmospheric concentrations of CO_2, aims at increasing the C content in the top 40 cm soil by 0.4 % annually by improved land management and land use change. One option to store more C in soil is to revert degraded land and arable soils low in C and with limited productivity into permanently vegetated soils such as semi-natural grasslands (Powlson et al., 2011). The conversion of arable soils to grassland may accomplish a rapid initial increase in soil C storage (Poeplau et al., 2011; Kämpf et al., 2016; Rosenzweig et al., 2016) and introduce a long-lasting gradual increase in soil C with new steady-state equilibriums reached only after > 100 years. However, other studies have found limited or no effect of converting arable soils to grassland (Don et al., 2009; Gosling et al., 2017).

In terms of soil C sequestration, the benefit of converting long-term arable soil into semi-natural grassland has two components: the increase in soil C ascribed to the grassland vegetation and the avoidance of soil C losses derived from a continuation of the arable management. However, changes in the C content of long-term arable soils are slow and experimentally verifiable only over extended periods. Combined with simulation models, long-term agricultural experiments, kept under well-defined management and with records of climate parameters, harvest yields and soil characteristics, represent a unique research platform for establishing the over-all benefit of converting arable soils into permanent grassland. With a long period under arable management, it is possible to establish a baseline change in soil C storage before introducing grassland.

In this study, we quantify changes in C and N in topsoil from the Sandmarken experiment (initiated 1894 at Askov Experimental Station, Denmark) before and after its conversion in

1998 into unfertilized semi-natural grassland. This long-term experiment is on a low productive coarse sand soil and includes replicated plots that until 1998 were subject to different fertilization. Archived soils sampled during 1942-2012 were analysed for C, N, ^{13}C and ^{15}N. Using measured harvest yields (1923-1997), topsoil C contents (1923-2012), allometric functions, and the C-TOOL simulation model, we evaluated changes in topsoil C and N content and C inputs in the arable (1923-1997) and grassland phase (1998-2012).

Materials and methods

The Sandmarken site

The Sandmarken long-term experiment is located in South Jutland, Denmark, close to Askov Experimental Station (55°28′N, 09°07′E). The site experiences an average annual precipitation of 862 mm and a mean annual temperature 7.7 °C (1961-1990 average). The soil is a coarse sand classified as Inseptisol (Orchrept) according to USDA Soil Taxonomy. The topsoil (0-20 cm) contains 4 % clay (< 2 μm), 4 % silt (0-20 μm), 34 % fine sand (20-200 μm), and 57 % coarse sand (200-2000 μm). The soil layers down to 100 cm have similar textural composition and bulk density (1.5 g cm^{-3}). Average annual atmospheric deposition of ammonia and nitrate at Askov Experimental Station has ranged from 8 kg N ha^{-1} during 1921-1927 to 16 kg N ha^{-1} during 1987-1989 (Grundahl and Hansen, 1990).

The arable rotation phase

The Sandmarken experiment consist of four blocks (termed G1-, G2-, G3- and G4-field), and during 1894 to 1997, it was under a four-course rotation of autumn-sown cereals, root crops, spring-sown cereals, and leguminous crops (Table 1) with each crop present every year in a separate field. Leguminous crops were whole-crop harvested in green condition, and cereals and root crops at maturity. All aboveground biomass components were removed at harvest leaving only short stubbles. The main treatments in the experiment were unfertilised and unmanured plots (0; termed unmanured), and plots amended with either animal manure (AM) or mineral fertilisers (NPK). In each field, the 0, AM and NPK treatments were represented by 2, 3 and 3 replicate plots, respectively. Table 1 shows the amount of total-N, total-P and total-K added in NPK and AM during 1894 to 1997. The rate increased in 1923, 1949 and 1973, but within a given period, NPK- and AM-treated plots always received similar quantities of total-N, P and K. Farmyard manure was applied in AM plots from 1894 to 1972, supplemented with liquid manure from 1923 to 1972. Since 1973, the AM treatment has relied on cattle slurry. Each treatment plot was 55 m^2 with the central 24 m^2 used for determination of harvest yields. Christensen et al. (1994) provides further details on the site, the experimental layout and fertilization treatments.

The grassland phase

Following crop harvest in 1997, nutrient additions ceased and the arable crop rotation converted into permanent grassland with perennial rye grass (*Lolium perenne* L.) and red fescue (*Festuca rubra* L.) sown in mid-March 1998. The grass was mowed once or twice each year with the cut biomass left on the soil plot. Thus, grass productivity was not determined. The Sandmarken has since 1998 been considered a semi-natural grassland with no fertilization and no removal of plant biomass. Mg-enriched lime was applied in 1997 (4 Mg ha^{-1}) before grass was sown and again in 2005 (3.5 Mg ha^{-1}) when grass was re-sown.

Soil and plant samples

Every four years since 1923, soil sampled from 0-20 cm depth of each treatment has been analysed for C concentration by dry combustion methods. Since 1942, dried soil samples have been archived. For soils collected in 1942 and 1953, only samples bulked across treatment replicates are available in the archive. For the present study, we relied on samples collected in the G1-, G2- and G3-field in 1942, 1953, 1964, 1976, 1985, 1996, 2000, 2004, 2008 and 2012. Soil subsamples were sieved (< 2 mm), ball-milled, packed into tin capsules, and analysed for total C, total N and for ^{13}C and ^{15}N natural abundance at the Stable Isotope Facility, University of California, Davis, USA, using dry combustion and isotope-ratio mass spectrometry. We applied the same analyses to plant samples collected in October 2011 and again in May 2012 from plots kept unfertilized (0) or dressed with AM during the rotation phase. Sampling took place in a 0.5×0.5 m^2 area located in the centre of each replicate plot.

Data for climate, crop yields and soil C concentrations

For the present study, we used historical temperature data recorded at Askov Experimental Station. Grain yields of spring- and autumn-sown cereals and total yields of leguminous and root crops for the period 1923-1997 were from the yield database associated with the Sandmarken experiment. Concentrations of C in topsoil were tabulated values reported for Sandmarken by Kofoed (1982) for the period 1923-1976, and results of present analyses of archived soil samples (see above).

C-TOOL simulations

We used allometric functions and the C-TOOL simulation model (Taghizadeh-Toosi et al, 2014a) to calculate and estimate C inputs to the topsoil and to simulate topsoil C contents during the period 1923-2012. The C-TOOL model consists of three conceptual pools (FOM: fresh organic matter; half-life 0.5 year, HUM: humified organic matter; half-life 20 years, ROM: resistant organic matter; half-life 1500 years) in topsoil (0-25 cm) and subsoil (25-100 cm) and simulates medium- to long-term soil C storage in mineral agricultural soils based on a limited number of input parameters. C-TOOL applies temperature-dependent first order kinetics to regulate C turnover and has previously been calibrated and tested for Danish agricultural soils under arable rotation and grassland (Taghizadeh-Toosi et al, 2014a; Taghizadeh-Toosi and Olesen, 2016).

The initialization of C-TOOL applied the C pool sizes from simulations based on measured data (1986, 1997 and 2009) from the Danish nation-wide C monitoring network (Taghizadeh-Toosi et al., 2014b; Taghizadeh-Toosi and Olesen, 2016). The model initial soil C content (year 1923) for each fertilizer treatment was established by minimizing the sum of squares between simulated and measured (1923-1996) soil C contents using the nonlinear curve–fitting function (lscurvefit) in MATLAB (MathWorks Inc. 2016). For that, the calculations of C inputs into the soil during the arable phase was based on records of crop yields (1923 to 1997) and allometric functions relating harvest yields to total above- and belowground biomass production. Calculations of the amount of C added with AM relied on manure dry matter contents and C concentrations.

The estimation of soil C input during the grassland phase (initiated 1998) was by running the simulations using measured topsoil C contents (2000-2012) and the nonlinear curve fitting in MATLAB to minimize the sum of the squared error of the difference between simulated and measured soil C contents. Then, the optimized C input for each treatment was used during the grassland phase, and C-TOOL was finally ran for the entire experimental period (1923-2012).

All measured soil C data refer to 0-20 cm soil depth. Since the topsoil simulated by C-TOOL refers to 0-25 cm depth, we made a proportional expansion of measured soil C concentrations to 0-25 cm during simulations. Subsequently, we back-transformed simulated values to 0-20 cm.

Statistical analysis

Concentrations of soil C and N in topsoil (0-20 cm) were converted to Mg C ha^{-1} and Mg N ha^{-1} using a bulk density of 1.5 g cm^{-3}. Changes in topsoil C content, N contents, $\delta^{13}C$, and $\delta^{15}N$ were analysed using mixed linear model with the procedure MIXED in SAS (SAS Institute 2008). We examined the relationship between $\delta^{13}C$ and C concentration and between $\delta^{15}N$ and N concentration using the linear regression function in SigmaPlot (Version 11). This tool was also used for graphics.

Results

Changes in topsoil C and N

Figure 1 shows the C and N concentrations in archived topsoils sampled during 1942 to 2012 from plots kept unmanured (0) or treated with animal manure (AM) or mineral fertilizer (NPK) during the arable phase. Throughout the period with arable rotation, C and N concentrations in unmanured soil remained lower than those in NPK soil, while AM treated soil showed the highest contents. Concentrations of soil C and N showed a constant and similar decrease, regardless of fertilization regime. The mean annual losses from the topsoil during this period correspond to 0.10 Mg C ha^{-1} and 0.012 Mg N ha^{-1} (Table 2).

When converted to unfertilized, semi-natural grassland in 1998, this trend was reverted. Now concentrations of C and N increased (Fig. 1), corresponding to a mean annual accumulation of 0.29 Mg C ha^{-1} and 0.017 Mg N ha^{-1} (Table 2). Topsoil C and N storage did not appear to approach any new steady-state levels after 14 years under grass and the ranking of pre-1998 fertilization treatments in terms of soil C and N contents remained in the grass phase (0 < NPK < AM).

Natural abundance of ^{13}C and ^{15}N

During the arable rotation phase, the topsoil increased in natural abundances of ^{13}C and ^{15}N (Fig. 1); the annual increase in $\delta^{13}C$ and $\delta^{15}N$ being 0.0021 ‰ and 0.014 ‰, respectively (Table 2). Although differences were not significant, the treatments seemed to rank differently for $\delta^{13}C$ (0 > NPK > AM) and $\delta^{15}N$ (AM > 0 > NPK). The apparent ranking of treatments in $\delta^{13}C$ and $\delta^{15}N$ remained after conversion to grassland, but now $\delta^{13}C$ and $\delta^{15}N$ values showed an average annual decrease of 0.063 ‰ and 0.074 ‰, respectively. Soil $\delta^{13}C$ related negatively to soil C content, while soil $\delta^{15}N$ and soil N content appeared unrelated

(Fig. 2). However, the relationship between C content and $\delta^{13}C$ differed for soils retrieved during the arable phase and the grassland phase. Within the arable phase, $\delta^{13}C$ increased 0.063 ‰ when soil decreased by 1 mg C g^{-1} soil; the corresponding value for soil collected during the grassland phase was 2.5 times higher (0.159 ‰).

Table 3 shows that the content of C and N and the ^{13}C and ^{15}N abundance in plant samples collected in the autumn 2011 and spring 2012 from soil plots kept unmanured or treated with manure during the preceding arable phase differed little. The only consistent difference was in $\delta^{15}N$. Plants grown on previously manured plots was higher in $\delta^{15}N$ (mean 0.7 ‰) than plants from unfertilized plots (mean -1.4 ‰).

Crop yields during arable rotation

Figure 3 shows the crop harvest yields obtained during 1923-1997 and used in the C-TOOL simulation. For NPK and AM plots, receiving similar inputs of nutrients, harvest yields were similar. The exception is autumn sown cereals (cereal rye) where plots dressed with NPK generally produced higher grain yields than AM plots. Crop yields obtained on unfertilized soils were consistently lower than those obtained on plots dressed with AM and NPK. This was most significant for leguminous and root crops; these rotation components, especially root crops, also showed the largest between-year variation.

C-TOOL modelling

Figure 4 shows the measured (averaged across fields and replicates) and simulated values for C and N content in the Sandmarken topsoil during 1923-2012. Carbon contents measured in this study on archived soils, sampled in 0, NPK and AM plots in 1942, 1952 and 1976, were similar to data reported by Kofoed (1982) for the same treatments and years. This confirms the reliability of the previous analytical protocols for soil C analysis and that the use of the combined dataset does not compromise the C-TOOL modelling work.

Measured changes in topsoil C stocks during 1923-2012 aligned well with values generated by the C-TOOL model regardless of fertilization regime although the best match was for unfertilized soil. The calculated average inputs of C to the soil using allometric functions during the arable phase averaged 0.41 Mg C ha^{-1} yr^{-1} (range 0.17 to 1.06) while inputs to NPK and AM treated soils were 1.37 (range 0.67 to 2.51) and 1.68 (range 0.63 to 2.85) Mg C ha^{-1} yr^{-1}, respectively. Inputs of C to the topsoil differed from year to year with the largest fluctuations found for AM treated soil. Under grassland, the C-TOOL simulated rather similar annual C inputs (3.21, 3.62 and 3.75 Mg C ha^{-1}) for soils previously supplied with AM, NPK or kept unfertilized, respectively.

Table 4 shows the distribution of topsoil C among the C-TOOL model pools. The pool of resistant organic matter (ROM; half-life = 1500 years) contained 13-14 Mg C ha^{-1} and remained almost constant during the period 1923-2012 with insignificant differences between nutrient treatments. The quantitatively most significant change was for C allocated to the pool of humified organic matter (HUM; half-life = 20 years). For soil kept unfertilized, HUM declined from 16.2 Mg C ha^{-1} in 1923 to 3.7 Mg C ha^{-1} in 1997. The decline in HUM was smaller for NPK and AM treated soils. The C contents in these soils changed from 16.5 and 17.4 Mg C ha^{-1} in 1923 to 7.6 and 10.0 mg C ha^{-1} in 1997, respectively. Following introduction of grassland, the amount of C in HUM increased again. The most conspicuous increase was for unfertilized soil. Here the HUM pool increased from 3.7 to 8.1 Mg C ha^{-1} after just 14 years under grass whereas changes in the pool of fresh organic matter (FOM; half-life = 0.5 years) and the ROM pool were insignificant.

Discussion

Generally, the harvest yields obtained in Sandmarken were very low when compared to current productivity in modern agriculture; this is especially true for cereal grain yields. The crops grown on Sandmarken were frequently drought-affected during the active growing period but the site was never subject to irrigation despite the very sandy nature of the soil. For autumn-sown cereals, the NPK treated plots generally produced higher grain yields than plots given equivalent amounts of N, P and K in animal manure. This probably reflects that during most of the arable period, NPK was applied late winter/early spring and animal manure in early autumn before planting of winter cereals. Thereby plots under AM treatment have experienced larger over-winter leaching losses of N, reducing the N available for subsequent crop uptake. The replacement of grass-clover in the arable rotation with lupines and later peas, both whole-crop harvested in green condition, increased the harvest yield on unmanured plots; however, this did not lead to higher C input to the topsoil.

The Sandmarken site belongs to one of the first areas brought into agriculture around the village of Askov. As evidenced by Land Register Maps dated 1793, Sandmarken had most likely been under ley-arable rotations for centuries before start of the fertilizer experiment in 1894. The low C and N concentrations reported by Kofoed (1982) for unmanured topsoil, sampled in 1914 (9.1 mg C and 0.7 mg N g^{-1} soil), confirm a long history of agricultural use. Nevertheless, the topsoil kept loosing C and N during the arable phase. Losses occurred at the same rate although the different treatments with different harvest yields, and thus different returns of crop residues to the soil, were reflected in the levels of C and N (0 < NPK < AM). The simultaneous loss of C and N meant that the C/N ratio differed little during the arable phase. The continuing loss of C and N in Sandmarken is in accordance with results from a number of other differently textured soils of long-term agricultural field experiments in Europe and North America, showing that loss of C and N from topsoil under arable rotations may continue for several decades (Christensen and Johnston, 1997; Johnston et al., 2009).

The good fit between measured C stocks and stocks calculated by the C-TOOL model confirms the ability of the model to reflect the longer-term dynamics of soil C. Most of the C loss during the arable phase was from the HUM pool with a half-life of 20 years with the pool of resistant C (ROM) remaining constant. For plots kept unmanured, the HUM pool decreased from 54 % of the topsoil C stock in 1923 to 22 % in 1997. Losses were smaller for C in plots treated with NPK and AM. During the same period, the ROM pool accounted for an increasing proportion of the C stored in the topsoil (43-44 % in 1923 to 57-77 % in 1997). The substantial increase in topsoil C initiated by the introduction of unfertilized semi-natural grassland was related to a considerable higher input of plant C, most of which accumulated in the HUM pool. Compared with rotations based on annual arable crops and conventional tillage, the absence of tillage may add to accumulation of C under permanent grassland although the quantitative effects of no-till on soil C accumulation are debated (Powlson et al., 2014). In Sandmarken, the increase in the HUM pool related to the soil C content at the time of land use change. For previously unmanured soil, the HUM increased 4.2 Mg C ha^{-1} while soils previously dressed with NPK and AM increased 2.7 and 1.3 Mg C ha^{-1}, respectively. Thus, the soil with the smallest C content showed the greatest potential for storing new C with medium turnover rate.

Changes in $\delta^{13}C$ measured during 1942-1996 corroborate the changed composition of topsoil C modelled with C-TOOL. Menichetti et al. (2015) found that topsoils retrieved during 1929-2009 from five European long-term bare fallow experiments (kept without C inputs for 27-80 years) showed a mean annual $\delta^{13}C$ increase of 0.008-0024 ‰ while losing 33-65 % of their pre-fallow C. The increased $\delta^{13}C$ was associated with an increased proportion of stable C in the soils (Barré et al., 2010). During the arable phase, we found a similar increase in $\delta^{13}C$ (0.0021 ‰) with a concomitant increase in the proportion of C ascribed to resistant soil organic matter. Although the fractionation between ^{12}C and ^{13}C that occurs during individual microbial turnover of organic matter is small, fractionations during subsequent multiple turnovers lead to accumulation of stable soil C enriched in ^{13}C. The decrease in topsoil $\delta^{13}C$ during the grass phase averaged 1 ‰ and is attributed the more negative $\delta^{13}C$ of the grass input but also a reduced decomposition rate shown by the accumulation of grass derived C in the HUM pool with medium turnover rate rather than in the FOM pool with short-term turnover rate. The $\delta^{13}C$ of grass sampled in 2011 and 2012 (-29.0 to -29.8 ‰) is 1-2 ‰ lower than $\delta^{13}C$ for arable crops grown in the Askov Lermarken site (Bol et al., 2005; Kanstrup et al., 2011). Similarly, Gregory et al. (2016) found more negative values for grass than for wheat plant material in a Rothamsted experiment. A selective accumulation of recalcitrant plant residue components low in ^{13}C (e.g. lignin and waxes) may have contributed to the decline in $\delta^{13}C$ that was introduced when the arable soil was converted into unfertilized grassland (Quideau et al., 2003).

Richardson et al. (2014) argued that long-term sequestration of C in soil requires a simultaneous sequestration of N to meet the stoichiometric requirements of the decomposer organisms and raised the question as to whether nutrient inputs would be required to generate new and stabilized C in the soil. Gosling et al. (2017) found no difference in topsoil C stocks between grasslands up to 17 years old and arable cropland and ascribed the lack of C accumulation under grassland to lack of available N resulting in low plant C input to the soil. However, the small differences in C input to the Sandmarken topsoils, subject to different fertilization during the arable phase, indicates that differences in initial soil fertility did not affect the productivity of this semi-natural grassland. Changes in topsoil N differed from changes in C and the C/N ratio of the soil organic matter increased from 8 in the arable phase to 17 in the grassland phase. During the grassland phase with no N_2-fixing legume component and no applied N, the sole source of N would be by atmospheric deposition. In the Askov area, wet deposition of ammonia and nitrate accounts for 16 kg N ha^{-1} yr^{-1}. This N input corresponds to the average annual increase in soil N measured during the grassland phase (17 kg N ha^{-1}), suggesting that little N was lost from the topsoil after conversion to grassland although the N input in dry deposition is not accounted for.

The establishment of a less leaky N cycle in topsoil under unfertilized semi-natural grassland corroborates our data on ^{15}N abundance. Because of isotope fractionations associated with losses of N by ammonia volatilization and by nitrification with nitrate subject to plant uptake, leaching or denitrification (Hobbie and Ouimette, 2009), soils with significant losses of N becomes enriched in ^{15}N. During the arable phase, the Sandmarken topsoil increased in $\delta^{15}N$ with soil subject to the AM treatment showing higher values than unmanured soil and soil taking NPK. The higher $\delta^{15}N$ value in AM soils is due to application of ^{15}N-enriched manure (Bol et al., 2005). In accordance with results from New Zealand reported by Stevenson et al.

(2010), the decrease in $\delta^{15}N$ observed after conversion into grassland indicates that losses of have been reduced and the N cycle in the soil has become tighter. However, plants grown in 2011 and 2012 still reflects the higher $\delta^{15}N$ value established in AM soil during the arable phase.

In terms of C sequestration, the benefit of converting arable soil into unfertilized semi-natural grassland has two components: increased storage of C ascribed to the grassland vegetation and elimination of losses of C associated with a continuation of the arable management. Ignoring the element of avoided C emission (or legacy effect) in studies of the effect of land use conversion may inflate estimates of the C sequestration potentials (Powlson et al., 2011; Smith, 2014). Long-term experiments with land use change offer a unique research platform to account for the legacy effect. During 75-years of arable management in the Sandmarken experiment, the average annual loss of soil C was 0.10 Mg ha^{-1}. This represents the legacy effect; the C loss that can be avoided. During the 14-years of unfertilized semi-natural grassland, the soil gained C at an average annual rate of 0.29 Mg C ha^{-1}. Thus, the over-all benefit of this change in land use from low-productive arable soil to unfertilized semi-natural grassland represents a C sequestration rate of 0.39 Mg C ha^{-1} yr^{-1}. For comparison, annual soil C sequestration in productive grasslands in Denmark rate can exceed 1 Mg C ha^{-1} (Christensen et al., 2009; Taghizadeh-Toosi et al., 2014b).

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Figure legends

Figure 1. Temporal changes of C content (a), N content (b), $\delta^{13}C$ (c) and $\delta^{15}N$ (d) in Sandmarken topsoil (0-20 cm). The treatments unmanured (0), mineral fertilized (NPK) and animal manure (AM) were applied only in the arable phase (see Table 1). The vertical dashed line showed the year of conversion of arable rotation to grassland (1998). Error bars indicate ± standard error of mean (SEM).

Figure 2. Topsoil $\delta^{13}C$ content versus total C content (a), and (b) soil $\delta^{15}N$ content versus total N content (b) in 0-20 cm soil in arable and grassland period. The treatments unmanured (0), mineral fertilized (NPK) and animal manure (AM) were applied only in the arable phase (see Table 1).

Figure 3. Grain yields for spring- and autumn-sown cereal grains and harvested biomass for leguminous and roots crops (Mg DM ha^{-1}) obtained in Sandmarken during 1923 to 1997 for crops grown on unmanured plots (0) and plots treated with mineral fertilizers (NPK) or animal manure (AM).

Figure 4. Lines show C-TOOL simulated and circles measured topsoil C stock (Mg C ha^{-1} in 0-20 cm). Black filled circles show data for soil C determined in this study, while grey filled circles show data retrieved from Kofoed (1982). Grey shaded areas represent the calculated annual C input into topsoil (Mg C ha^{-1} yr^{-1}).

Table 1. Crop rotation, amount of fertiliser (NPK) and animal manure (AM) application in Sandmarken from 1894-1997.

Period	Rotation elements				AM				NPK		
	Autumn sown cereals	Root crops	Spring sown cereals	Legumes	N	P	K	M [e]	N	P	K
					Kg nutrient ha^{-1}			Mg ha^{-1}	Kg nutrient ha^{-1}		
1894-1922	Rye	Mangolds and potatoes [a]	Oats	Grass-clover	42	13	31	9	40	13	27
1923-1948	Rye	Turnips and potatoes [a]	Oats	Grass-clover; Lupines [c]	71	16	65	10+4	70	17	70
1949-1972	Rye	Turnips or potatoes [b]	Oats	Lupines; Peas [d]	93	20	58	10+4	70	18	66
1973-1997	Rye	Turnips or potatoes [b]	Barley	Peas	98	19	87	25	100	19	87

[a] Plots divided into two subplots growing two crops simultaneously
[b] Turnips in every second rotation
[c] Lupines since 1943
[d] Peas since 1968
[e] Anmial manure given of wet weight. From 1894-1922, farmyard manure was applied. From 1923-1972, farmyard manure was supplemented with liquid manure. Since 1973, cattle slurry has been applied.

Table 2. Multiple linear regression for soil C, N, $\delta^{13}C$ and $\delta^{15}N$ (treatment fixed effect). Regressions were performed separately for the arable phase (1942-1996) and grassland phase (2000-2012). The slope 1 values represented slope for each treatment, and the slope 2 values showed slope ± standard error of mean for all treatments together. Slope 2 were statistically significant at $p<0.05$ level.

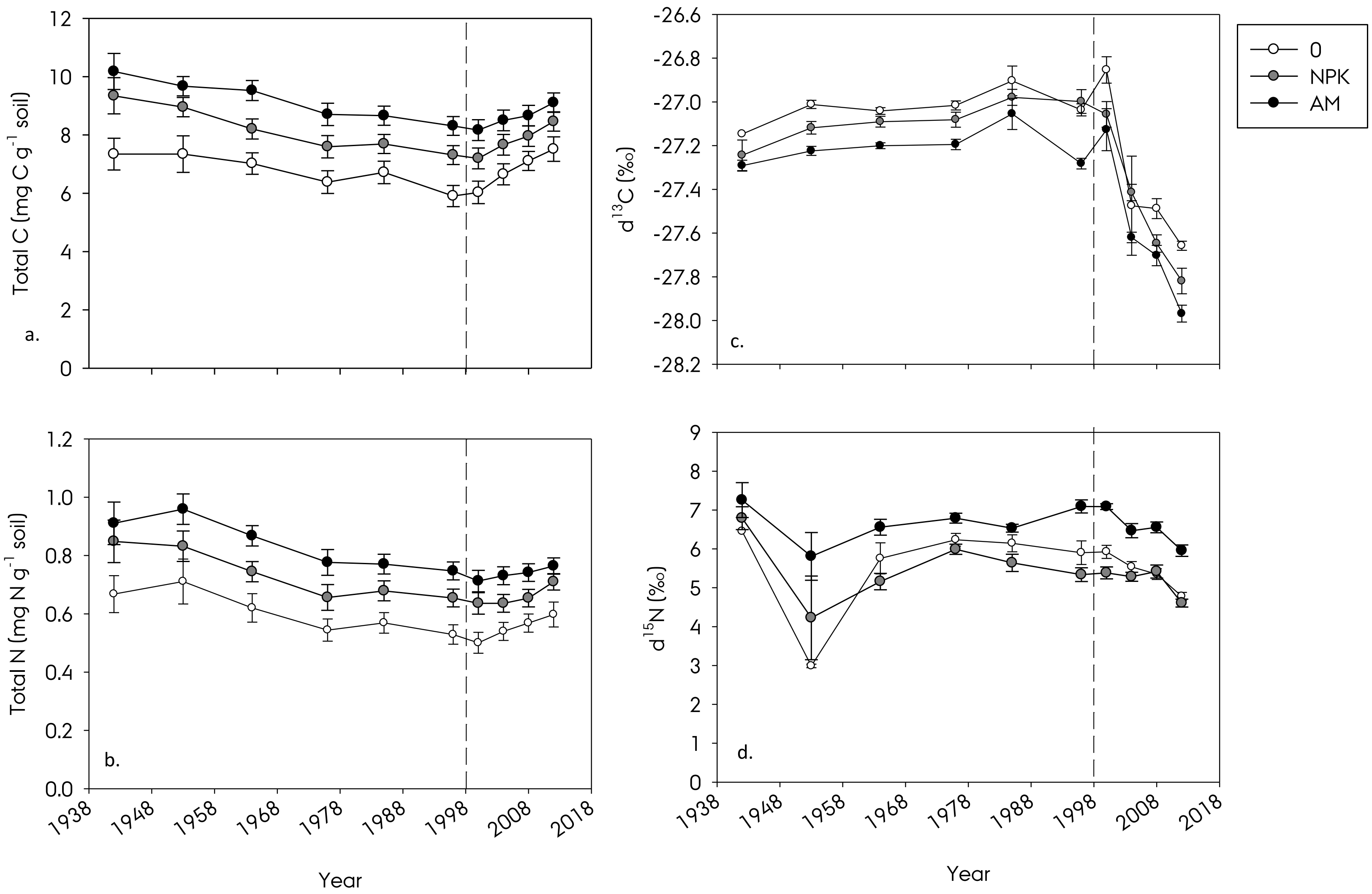
Target items	Treatment	Arable land period (1942-1996)		Semi-natural grassland period (2000-2012)	
		Slope 1	Slope 2	Slope 1	Slope 2
C (Mg C ha^{-1})	0	-0.09	-0.10 ± 0.01	0.37	0.29 ± 0.05
	NPK	-0.11		0.31	
	AM	-0.10		0.22	
N (Mg N ha^{-1})	0	-0.010	-0.012 ± 0.002	0.024	0.017 ± 0.005
	NPK	-0.011		0.018	
	AM	-0.012		0.012	
$\delta^{13}C$ (‰)	0	0.0013	0.0021 ± 0.0007	-0.061	-0.063 ± 0.005
	NPK	0.0039		-0.063	
	AM	0.0008		-0.065	
$\delta^{15}N$ (‰)	0	0.033	0.014 ± 0.006	-0.090	-0.074 ± 0.010
	NPK	0.003		-0.055	
	AM	0.014		-0.083	

Table 3. Carbon and nitrogen isotopic compositions and contents in plants sampled in autumn 2011, and in spring 2012 from plots kept unmanured (0) or treated with animal manured (AM) during the arable phase. The values showed as mean ± standard error of mean. Lower case letters indicate significant differences between treatments at *p*<0.05 level.

Treatments	$\delta^{13}C$ (‰)	C content (%)	$\delta^{15}N$ (‰)	N content (%)
Autumn 2011				
0 (n=6)	-29.0 ± 0.2^{a}	41.7 ± 0.7	-1.3 ± 0.4^{b}	1.5 ± 0.1
AM (n=9)	-29.8 ± 0.1^{b}	42.3 ± 0.6	0.6 ± 0.4^{a}	1.6 ± 0.1
Spring 2012				
0 (n=6)	-29.2 ± 0.1	43.4 ± 0.1	-1.5 ± 0.4^{b}	1.4 ± 0.1^{b}
AM (n=9)	-29.4 ± 0.1	43.5 ± 0.1	0.8 ± 0.4^{a}	1.6 ± 0.1^{a}

Table 4. Simulated C-TOOL pools (0-20 cm) in the first year of simulation (year 1923), at the end of the arable phase (1997), and at the first year (year 1998) and the end (year 2012) of the grassland phase. The FOM, HUM, and ROM indicated fresh, humified and resilient organic matter, respectively.

	0			NPK			AM		
Year	FOM	HUM	ROM	FOM	HUM	ROM	FOM	HUM	ROM
1923	0.8	16.2	13.1	1.4	16.5	13.3	1.2	17.4	14.1
1997	0.1	3.7	12.9	0.6	7.6	13.2	0.5	10.0	14.0
1998	1.3	3.9	12.9	1.5	7.8	13.2	1.3	10.0	14.0
2012	1.5	8.1	12.9	1.4	10.5	13.1	1.3	11.3	14.0



Total C (mg C g-1 soil)
a.
Total N (mg N g-1 soil)
b.
d13C (‰)
c.
d15N (‰)
d.
0
NPK
AM
Year
Year

Figure 1.

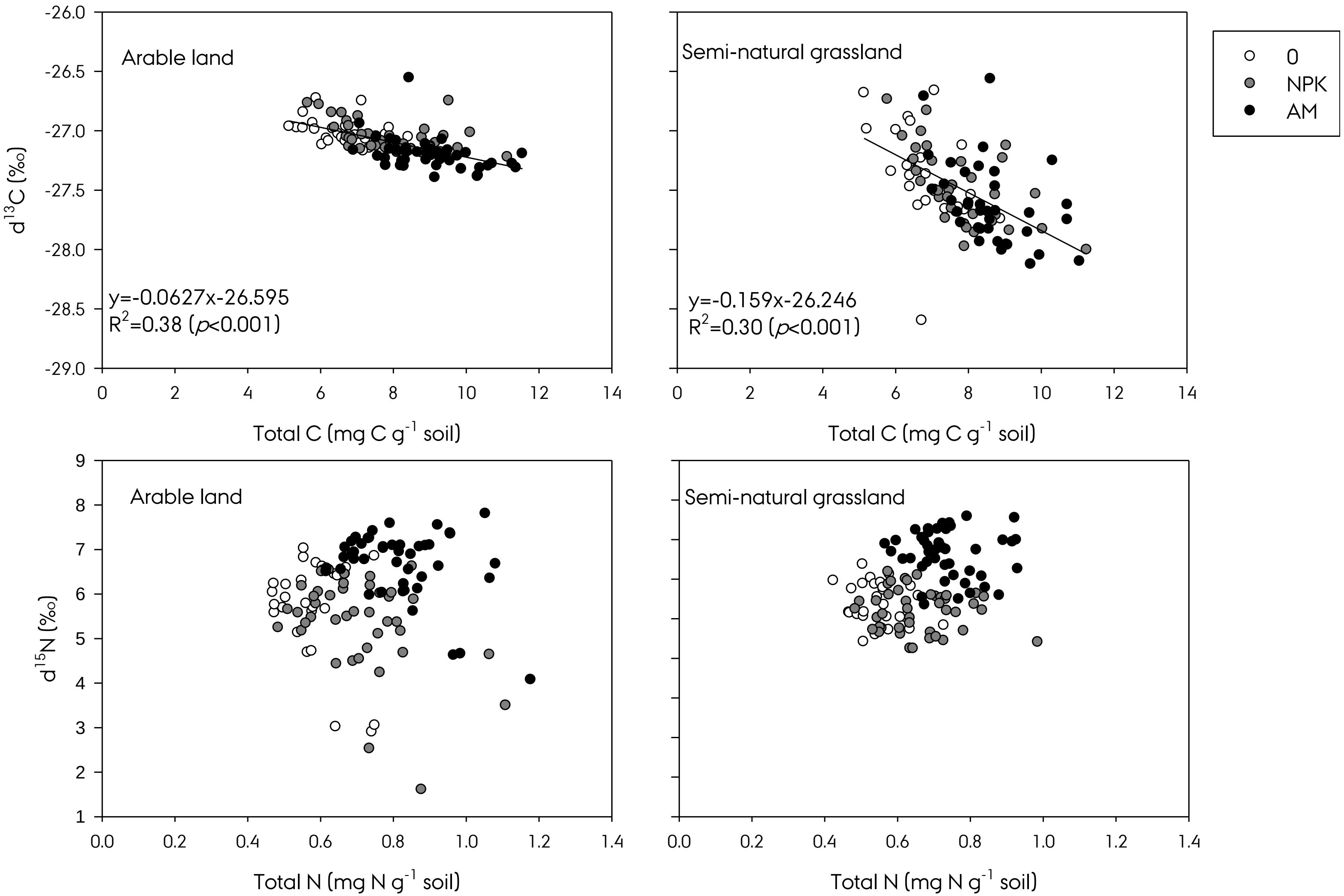

Arable land
y=-0.0627x-26.595
R^2=0.38 (p<0.001)
d^{13}C (‰)
Total C (mg C g^{-1} soil)
Semi-natural grassland
y=-0.159x-26.246
R^2=0.30 (p<0.001)
0
NPK
AM
Arable land
d^{15}N (‰)
Total N (mg N g^{-1} soil)
Semi-natural grassland
Total N (mg N g^{-1} soil)

Figure 2.

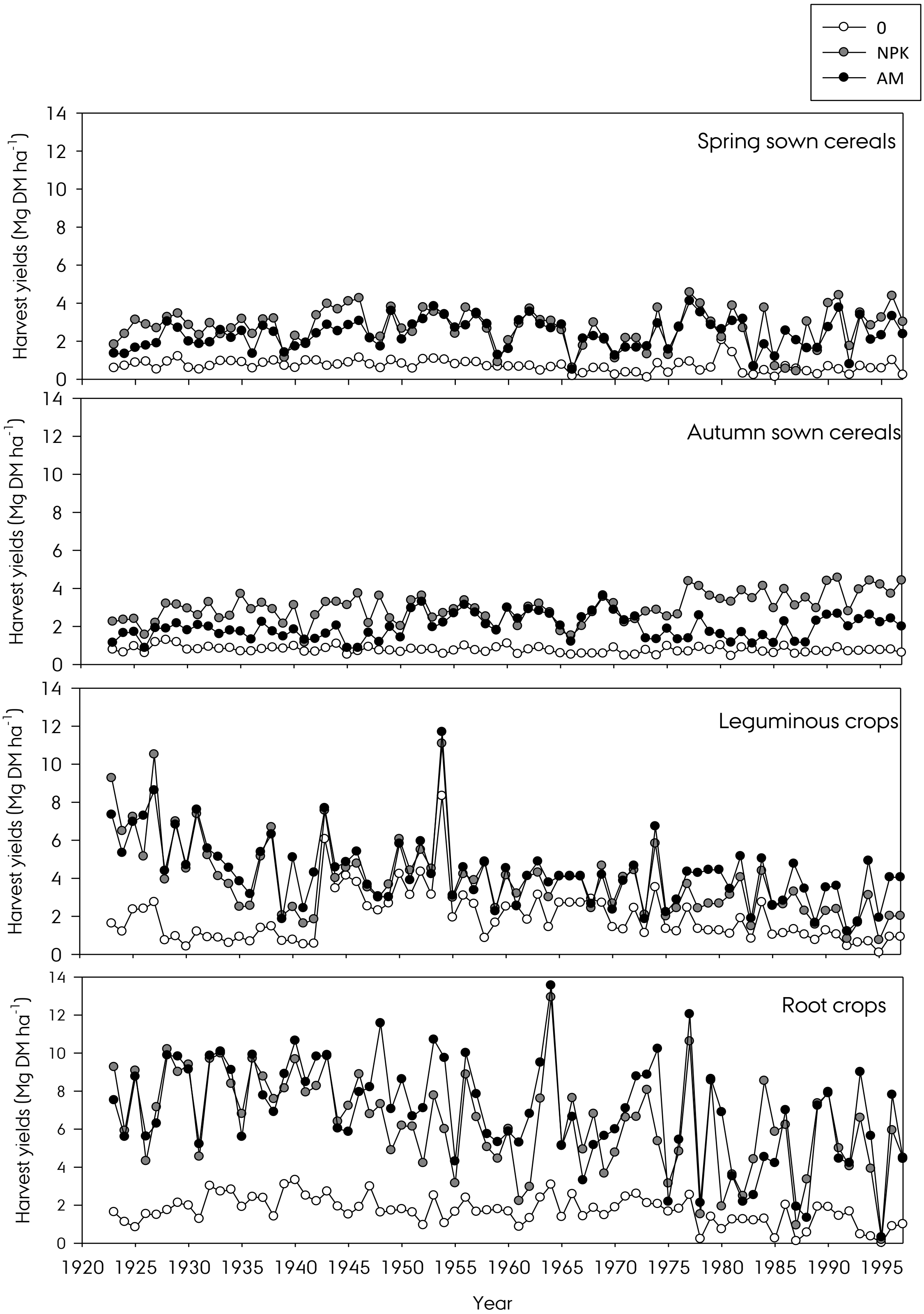

0
NPK
AM
Spring sown cereals
Autumn sown cereals
Leguminous crops
Root crops
Harvest yields (Mg DM ha^{-1})
0
2
4
6
8
10
12
14
1920 1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995
Year

Figure 3.

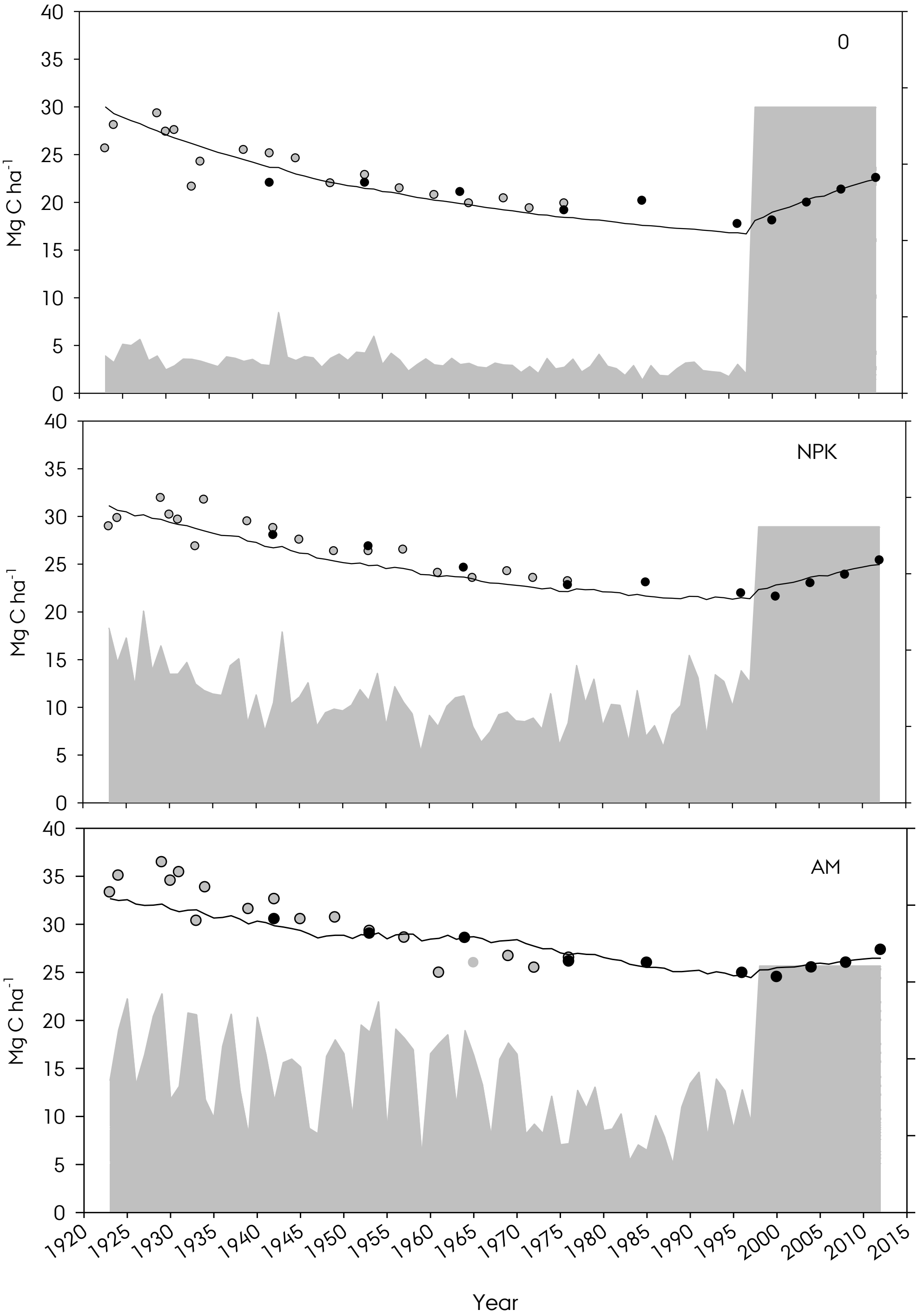


Figure 4.