SmartSOIL

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Report and publication on meta-analyses and new experiments of crop yield to soil functions

Author and co-authors:

Report prepared and summarized by Kirsten Schelde, Aarhus University, Denmark

Co-authors: Please refer to the author lists of the manuscripts. All individuals are co-authors of this deliverable report.

Executive summary

This report compiles the results of work carried out in the SmartSOIL task 1.1. Several activities in the task contributed to explore the crop yield responses to soil C flux and stock components. Hence, the outcome is not one, but four scientific papers. The manuscripts have not been submitted at the time of submission of this deliverable report but they will be submitted for review during 2014:

The first paper "Soil carbon content as affected by management and climate in Europe" describes the results of a statistical analysis of data from the database of Long Term Experiments (LTEs) established in the SmartSOIL project. The paper shows that it was possible to model and predict development in soil carbon over time as a function of climate (soil temperature), carbon inputs (straw, organic manure, crop residues), crop rotation (crop types), and tillage intensity (conventional tillage or not).

The second paper "Crop yield and N utilization as influenced by C flux and stock components: a European multi-site experiment" describes the new experiments made at three locations in Europe in order to explore the concept of C flows and C stocks in trying to isolate the effect of management from those of soil organic matter content. The approach surmises that flows and stocks play separate and different roles in relation to soil properties, soil functions and crop productivity, and that soil management can be designed to optimize either flow-derived or stock-derived functions. The paper shows that wheat responded differently to the experimental factors analyzed, depending on the experimental site. N fertilization rates affected the accumulation of both grain and straw final dry mass at Askov (the Danish site) and Pisa (Central Italy) but not at Foggia (South Italy). The effect of carbon stock was significant only at Askov and for grain yield that increased in high carbon stock conditions. Carbon flows negatively influenced the accumulation of grain and straw at Pisa and Foggia, while at Askov only straw was slightly reduced in high carbon flow conditions. Some of the differences in responses at the different sites can possibly be related to differences in carbon stocks between treatments, which were higher for Askov compared to the Italian sites.

The third study ("Arbuscular mycorrhiza fungi as soil health indicator") aimed to investigate the possible link between carbon sequestration and soil health. It relied on soil samples taken from the same locations as reported in the second paper described above (i.e. Askov, Pisa, and Foggia sites). The objective was to study the influence of incorporation of organic matter, mechanical soil management, and crop rotation on soil health as measured by presence of a soil health indicator organism; arbuscular mycorrhizal fungi (AMF). Therefore soil and wheat roots were sampled in the long term experiments in order to evaluate influence of different management strategies and of carbon sequestration on AMF inoculum potential of the soil, as an expression of soil health. Overall, the work showed a positive correlation between amount of incorporated straw and AMF inoculum potential of the soil, revealing that straw incorporation increased both soil health and the soil microbial carbon pool.

Finally, the fourth paper, "Crop yield responses to input intensity under varying soil carbon stocks and flows", resulted from a re-analysis of data previously published in two papers. The experimental approach of the studies allowed exploring and possibly distinguishing effects of SOC on crop productivity that are beyond effects of indirect N supply to the crop. The main results of the analyses were that for one of the sites (JYN), cereal yields and crop physiological nitrogen utilization efficiency (NUE) seemed to result from N supplies (N uptake) alone. However, according to a second dataset (ASK), there was an additional trend in the cereal yield and NUE that was significantly correlated with soil organic carbon (SOC) content. For individual high N fertilizer rates at the ASK site, high N uptake rates were associated with relatively higher NUE at locations where SOC was also relatively high. The trend was significant and relevant from an agronomic point of view: an improved soil C status by 1% SOC could increase the yield by approximately 10% compared to a mean yield. The analyses could however only indicate, not prove, that SOC caused the advantageous NUE.

The main conclusions based on analyses of the experiments were:

- Changes in soil carbon stocks can, based on LTEs, be well explained from crop choice and crop management, primarily related to above and below ground inputs.
- Soil carbon stocks are linked to soil nitrogen stock and to supply of nitrogen to the crops
- Addition of carbon rich materials can in the short term lead to yield reductions resulting from immobilisation of nitrogen
- Some, but not all, experiments show increases in yield potential and/or N use efficiency at higher soil carbon stocks

Soil carbon content as affected by management and climate in Europe

Multi-author** manuscript led by S. Baby*

**Dept of Agroecology, Aarhus University, 8830 Tjele, Denmark*

*** Authors representing all LTE sites included in the study, and additional key researchers from SmartSOIL*

Introduction

Soil and crop management affects soil C stocks and hence are important soil functions. There is still something to be learned from the long-term experiments if we gather them to focus on management effects on carbon changes across the climates of Europe.

The aim of this analysis is to study the long term effects of different management practices on soil carbon across Europe. The effects of various inputs, cropping systems, tillage effects, temperature effects and influence of soil type are investigated.

European long term experiments (LTEs) were earlier compiled in a database (EuroSOMNET; Smith et al. 2002; Franko et al. 2002) but a comprehensive meta-analysis like the one made here does not seem to have been made.

Materials and Methods

To analyse the long term effects of different factors in soil organic carbon, data was collected from LTEs with different treatments located in eight European countries. The collected data contains information about organic inputs, fertilizer inputs, soil carbon contents, crop management, harvest yield, and climate. A subset of the collected data was chosen for the analysis, satisfying the following two criteria: 1) The experiment spans at least 25 years 2) There are at least 3 soil organic carbon sampling measurements. An overview of the selected experiments is given in Table 1.

Table 1. Summary of the data for the analysis

Country	No.of Treatments	Exp. Start	Last sampling	Exp. Status
Denmark(Askov)	3	1929	2008	On going
Denmark(Fallow)	3	1956	1986	Completed
UK(BBK)	5	1843	2005	On going
UK(HFB)	4	1852	1998	On going
UK(Park Grass)	3	1876	2009	On going
Belgium	6	1959	1994	On going
Germany(Fallow)	4	1984	2009	On going
Germany(Model)	4	1984	2009	On going
Germany(Static)	6	1956	1990	On going
Italy(Foggia-fr1)	6	1983	2009	On going
Russia(Torzhok)	5	1956	2003	Completed
Russia(Ssh)	7	1960	2006	On going
Sweden(ORJ)	4	1957	2007	On going
Sweden(ULT)	3	1956	2003	On going

Summary of treatments

The treatments in the experimental data vary in the type and amount of C inputs, crops, tillage etc. We give a country-wise summary of the experiments below.

Denmark

Two experimental data sets with six treatments are included from Denmark. The first experimental set contains three treatments in terms of carbon and fertilizer inputs: no organic inputs and NPK, farm yard manure input without NPK and only NPK inputs. The cropping system followed a four-year crop rotation: winter cereal, root crop, spring cereal and grass-clover. Winter cereals were winter wheat since 1949. Between 1923 and 1949, winter cereals were winter rye or winter wheat. Spring cereals were spring barley since 1949. During 1923-1948, spring cereals were oats or barley. The second experimental set consists of three fallow treatments without organic inputs and varying N inputs (0, 70 and 100 kg).

UK

Three experimental data sets from UK are included in the analysis. The first experiment (Broadbalk) contains 5 treatments with varying levels of fertilizers and organic inputs: No fertilizers or manure, only PK, NPK, 35 t/ha manure with N from 1968, 35t/ha manure without any fertilizers. All treatments are applied on winter wheat. The second experiment is done with spring barley with four similar treatments as in the previous one: No fertilizer or manure, NPK, manure, manure applied from 1871-1985. In all treatments plots were divided into 4 subplots from 1968 and was given 0, 48, 96 or 144 kg N/ha each year, in rotation. The third experiment contains three treatments with permanent grass: no fertilizers or manure, manure, and NPK.

Belgium

Treatment differences for experiments from Belgium are in how crop residues and manure are applied. There are six treatments in this experiment: Removal of crop residues, Ploughing in crop residue and pig slurry and lime addition, Removal of crop residues and pig slurry and lime addition, farm yard manure addition and crop residues removal, ploughing in straw but removal of green products and green manure application with ploughing in of crop residues.

Germany

Three sets of experiments from Germany are considered in the analysis. The first experiment contains different levels of FYM application to bare soil: no FYM, 50t/ha, 100t/ha and 150t/ha. In the second experimental set the same treatments are applied to different crops in rotation (sugar beet, maize, winter wheat and potato). The last experimental set contains six treatments which in mineral fertilization and the amount of organic fertilizer input: No NPK or FYM, only NPK, 20 t/ha FYM in alternate years, 20t/ha FYM in alternate years with NPK, 30 t/ha FYM and 30t/ha FYM with NPK.

Italy

Treatments from Italy are all applied to drum wheat and they differ in how straw is added. The treatments are: no straw(T1), straw incorporation(T2), straw incorporation with 50 kg/ha N on residues(T3), straw incorporation with 100 kg/ha N on residues(T4), straw incorporation with 150 kg/ha N on residues(T5), treatment T3 with 500 m3/ha of water on residues, treatment T4 with 500 m3/ha of water on residues, treatment T5 with 500 m3/ha of water on residues and incorporation of crop residues without addition of N on residues. The added straw in these treatments amounts to 4t/ha.

Russia

There are 5 treatments in the first experimental set from Russia: control, 5 t/ha FYM, mineral NPK equivalent to 5t/ha FYM and 5 t/ha FYM with mineral NPK equivalent to 5t/ha FYM. The crops involved are spring barley, spring wheat, flax, potato and grass in rotation. The second set of experiments contains seven experiments: FYM application(3t/ha) with NPK (Treat 1), Treat1 with herbicides, Treat1 with herbicides, fungicides and retardants, NPK only (Treat 2), Treat 2 with herbicides, Treat 2 with herbicides, fungicides and retardants and a control treatment.

Temperature as a rate-modifying factor

The decomposition of carbon depends on soil temperature. For every 10 °C rise in temperature, decomposition generally increases one-to two-fold. We used a temperature effect F_T following Kirschbaum (1995) and Petersen et al. (2005), where T is the temperature (°C). The temperature effect equals unity at 10 °C:

$$F_T(T) = 7.24 \exp\left[-3.432 + 0.168\, T\, \left(1 - 0.5\frac{T}{36.9}\right)\right]$$

Soil temperature was approximated with the air temperature. The input temperature was the mean monthly air temperature. After calculating monthly responses the mean response for the year was calculated.

Statistics

Carbon dynamics were modelled using the following statistical model

$$C_t = C_{t-1} + e^{k_s N F_T} + \left(\sum_i b_i M_i + \sum_j d_j P_j\right) e^{0.5 k_s N F_T} + dL$$

Where

C_t is the measured soil carbon at sampling time t (t ha^{-1})

C_{t-1} is the measured soil carbon previous to C_t (t ha^{-1})

k_s is a decay parameter to be estimated and depends on soil type (clay content)

N is the number of years between sampling times t and t-1

b_i ‘s are the effects of C inputs from FYM and straw

M_i ‘s denote the amount C input through FYM or straw (t ha^{-1})

d_j ‘s are the effects of different crops

P_j’s are the number of seasons with different crop types: fallow, grass, or cereal and root crops. Since it was not possible to distinguish the effects of root and cereal crops they are combined into a single a single variable, CerealRoot.

d is the effect of no-tillage.

L is the number of years without tillage

In the model, two soil classes have been defined based on the clay content (below and above 15 % clay). For each soil class, a separate decay coefficient was estimated.

The decay rate for carbon inputs was taken to be a fraction of the decay time of the soil carbon. This fraction was estimated to be approximately 0.5 by simulation and was not estimated during model calibration.

Results and discussion

The following table gives the estimated parameters. The different decay rates for different soil types justifies having different soil types in the model.

	Estimate	Std. Error	t value	Pr(>|t|)	Significance
decay1	-0.010	0.001	-13.57	0.000	***
decay2	-0.004	0.001	-7.94	0.000	***
fym	0.210	0.003	65.95	0.000	***
straw	0.158	0.019	16.67	0.000	***
fallow	-0.462	0.042	-11.05	0.000	***
grass	0.323	0.043	7.52	0.000	***
CerealRoot	0.040	0.017	2.32	0.020	*
notill	0.064	0.020	3.18	0.002	**

The observed C values and the model predicted values are shown in Figures 1-12.

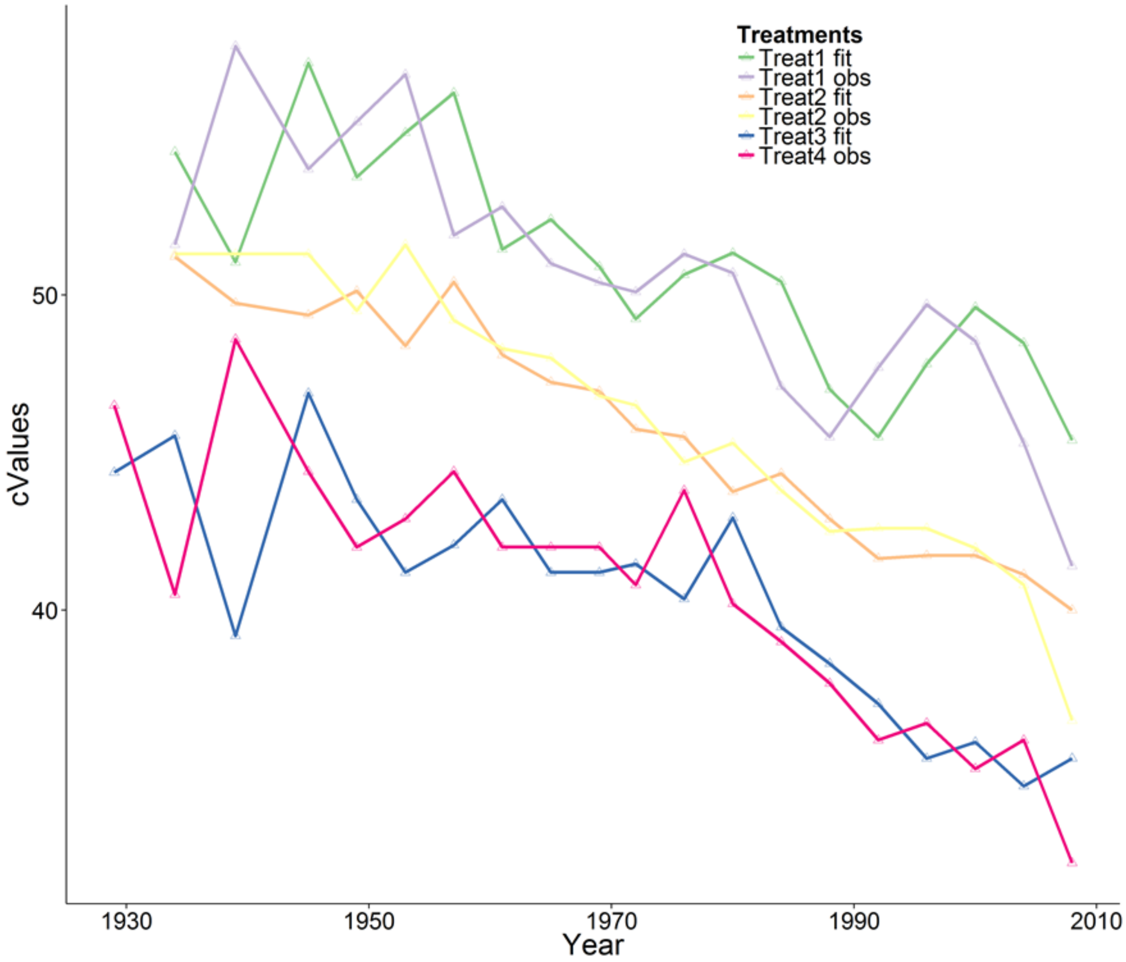

Figure 1. Observed and model predicted soil carbon for data from Askov, Denmark

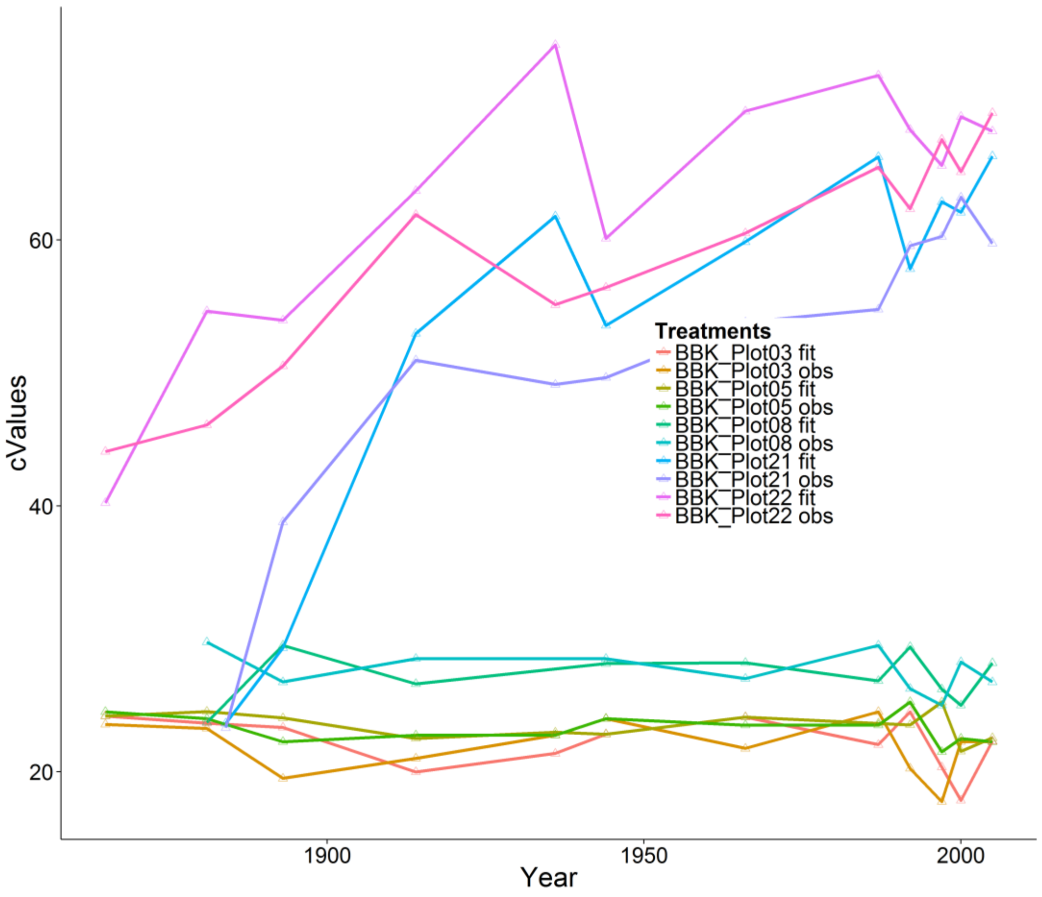

Figure 2. Observed and model predicted soil carbon for data from Broadbalk, UK.

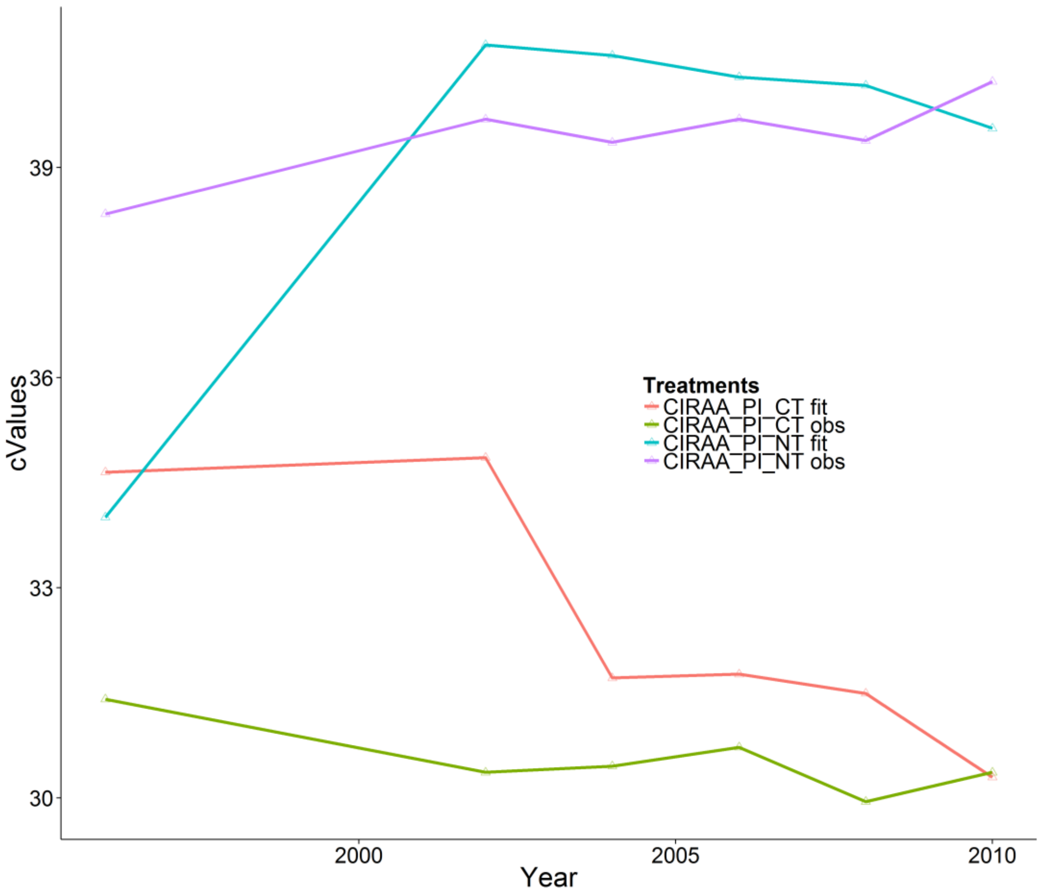

Figure 3. Observed and model predicted soil carbon for data from Pisa, Italy.

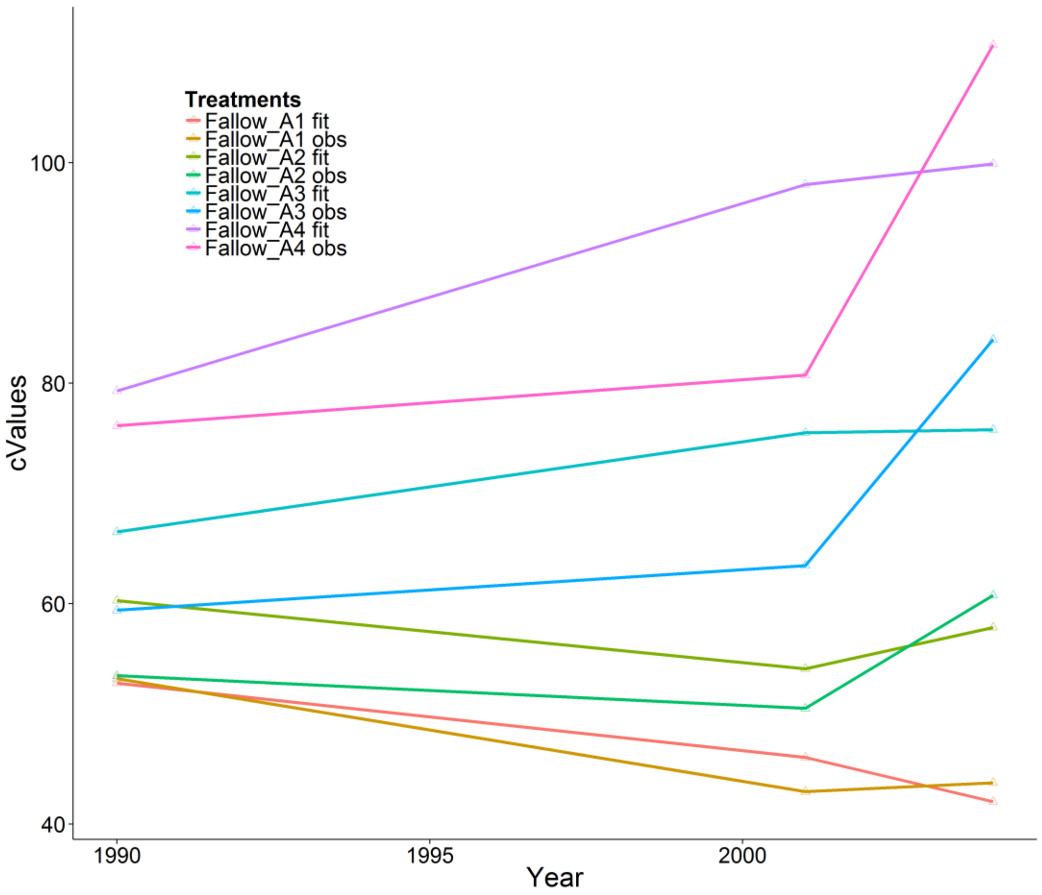

Figure 4. Observed and model predicted soil carbon for the fallow experiment from Germany.

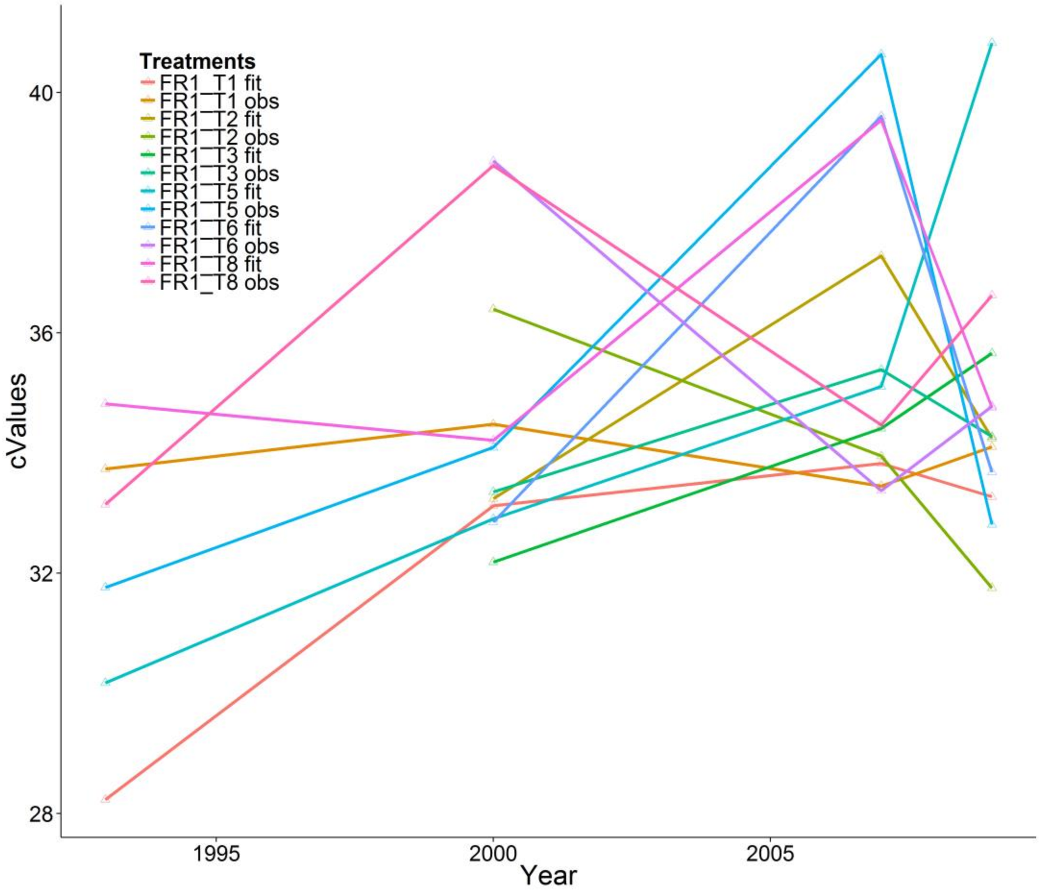

Figure 5. Observed and model predicted soil carbon for data from Foggia, Italy.

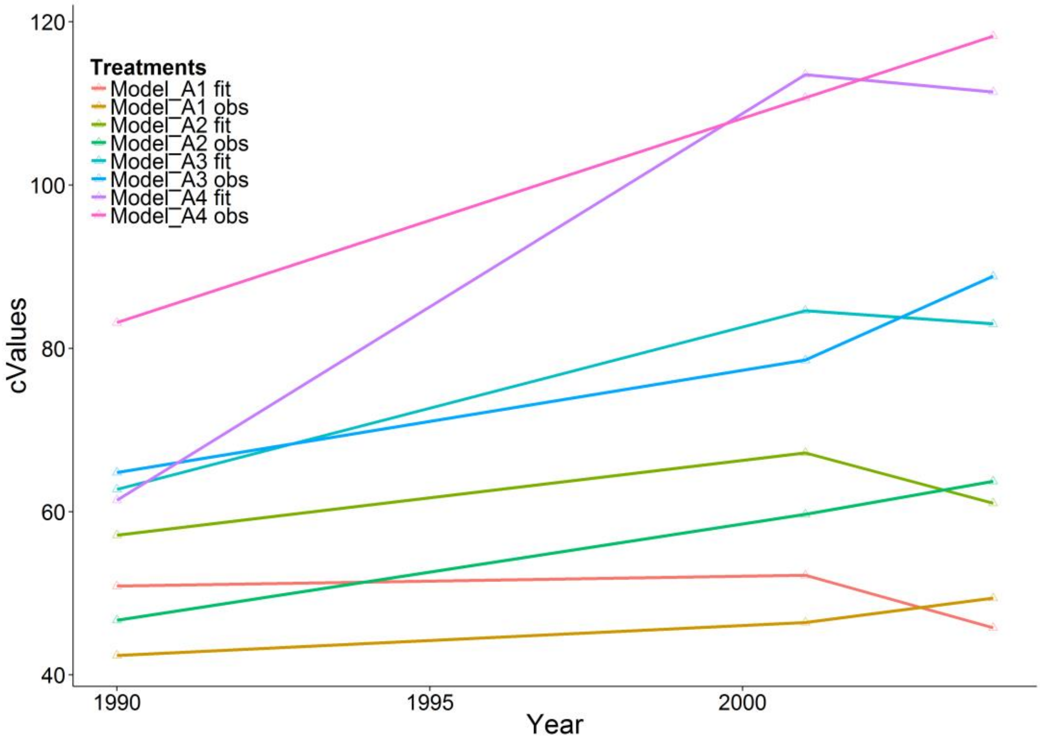

Figure 6. Observed and model predicted soil carbon for Bad Lauchstadt, Germany.

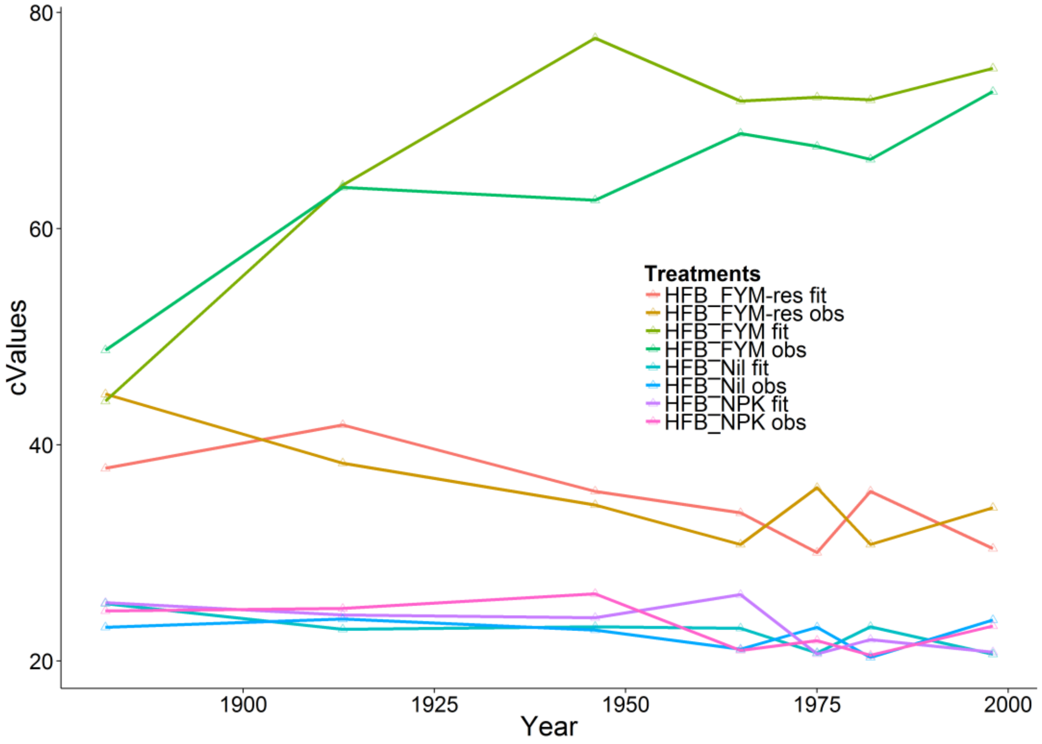

Figure 7. Observed and model predicted soil carbon for the data from the Hoosefield barley experiment, Rothamsted, UK.

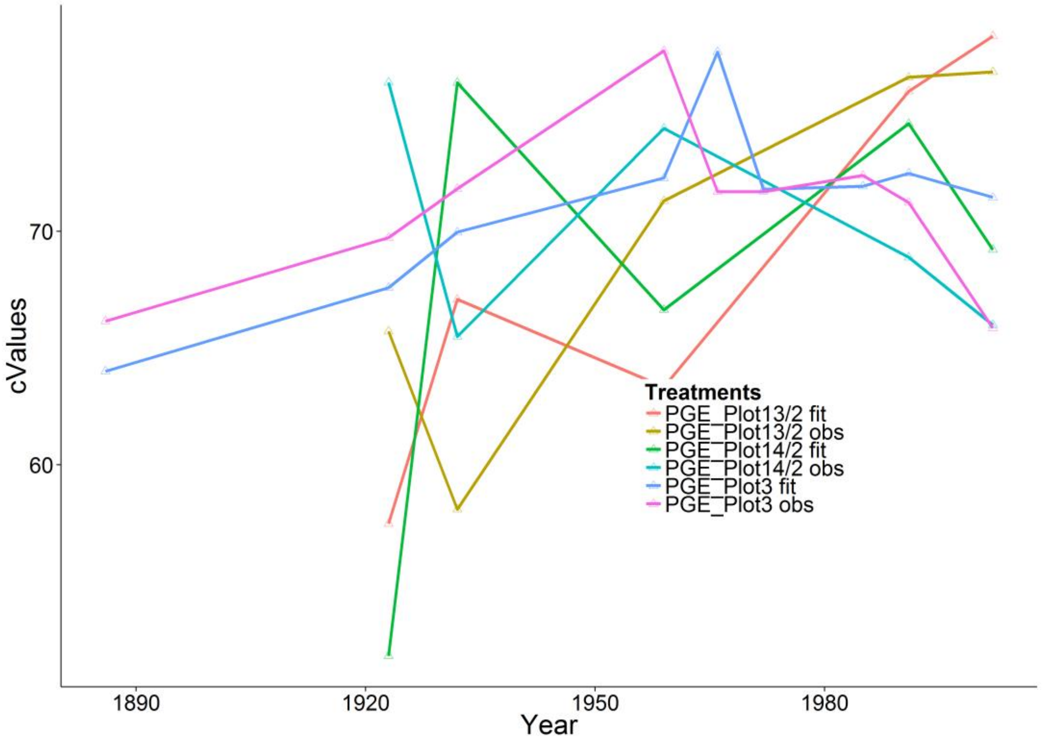

Figure 8. Observed and model predicted soil carbon for the Park Grass experiment at Rothamsted, UK.

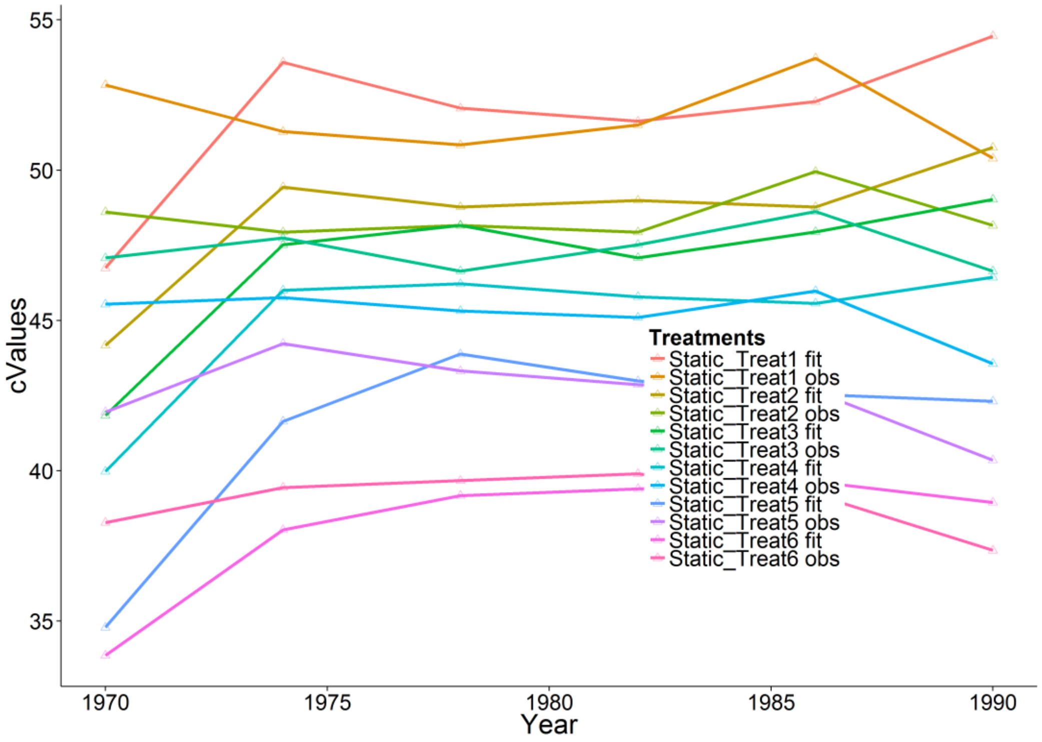

Figure 9. Observed and model predicted soil carbon for the experiment with varying levels of FYM from Germany.

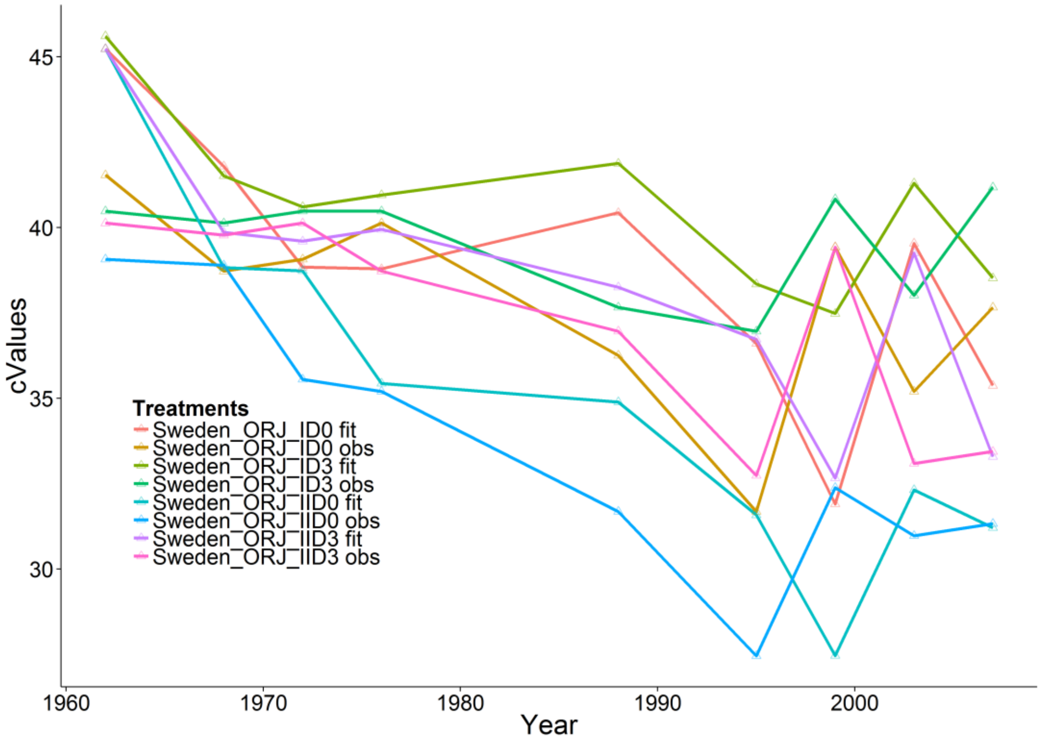

Figure 10. Observed and model predicted soil carbon for the data from Sweden.

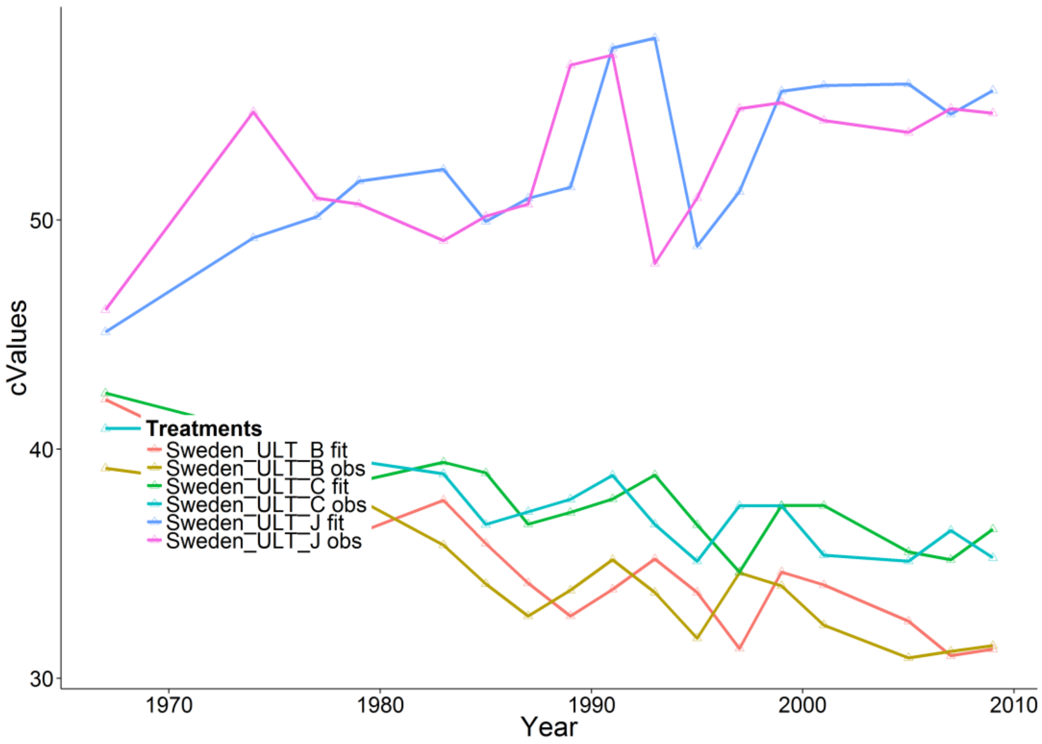

Figure 11. Observed and model predicted soil carbon for the second experimental data from Sweden.

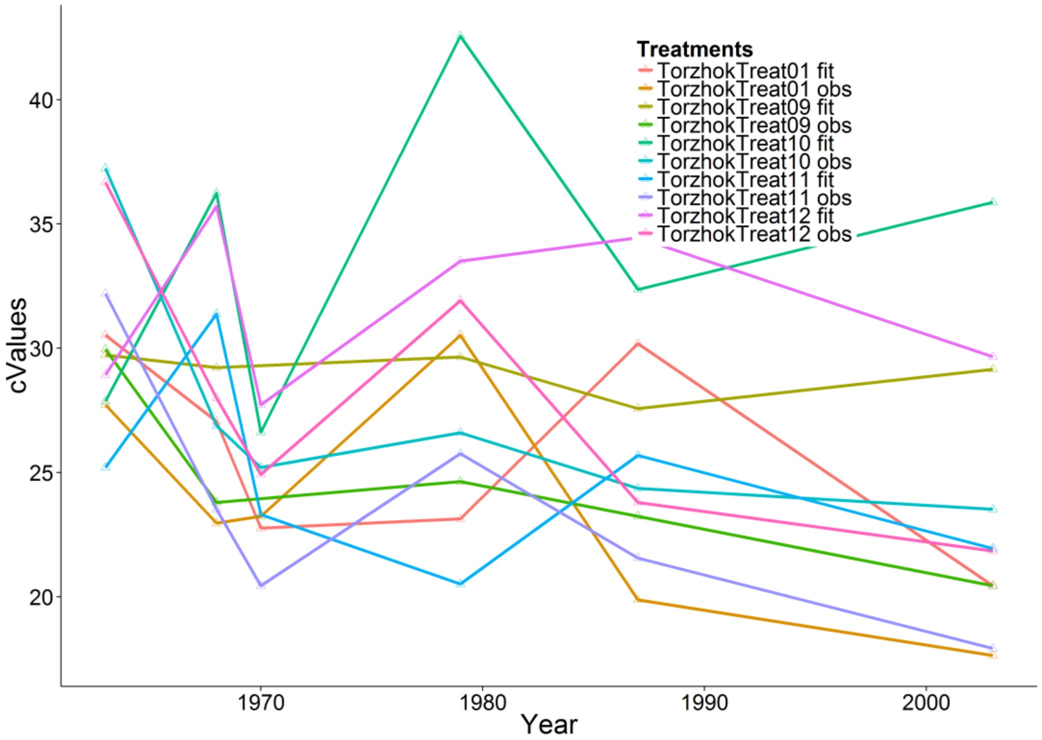

Figure 12. Observed and model predicted soil carbon for the data from Torzhok, Russia.

(Fragments for introduction and/or discussion section)
Soil carbon decomposition is controlled by soil microclimate variables, such as moisture, temperature, and oxygen content; by intrinsic soil properties, such as soil texture and pH; and by the quality of the organic substrate available to the decomposers. Temperature directly affects decomposition through its effects on microbial activity. In general, decomposition rates are highest when the temperature is between 25 °C and 35 °C and about 60% of the total soil pore volume is filled with water. Soil carbon decomposition functions best in soils with a pH that is close to neutral. Water affects decomposition through its controls on activity and transport of soil microorganisms, solubilization of organic constituents, oxygen supply, and soil pH. An optimum water content for decomposition is within a range that is not too high to limit oxygen availability and that is not too low to limit substrate availability and mobility of soil microorganisms. The ideal soil moisture condition for decomposition is between -10 and -50 kPa (or 30–60% water-filled pore space). Fluctuating moisture conditions in soil are common, but they do not necessarily have a negative impact on the ability of microorganisms to respond quickly to available substrates after rewetting (Franzluebbers 2005).

Decomposition rates tend to decline if soil moisture levels are too low or too high. The optimum is approximately equal to the moisture content at field capacity (the point at which all of the larger soil pores have drained due to the force of gravity, and water is held in the smaller capillary pores due to the matric forces in the soil). Soil microbes can function at soil moisture contents that are too low for plant growth but decomposition rates are reduced dramatically in wet, anaerobic soils (Cambardella, 2005)

Conditions of limited soil water availability reduce the rate of microbial activity due to the emergence of conditions of microbial water stress due to dehydration, and to the reduction in the size of the water films coating the soil grains. Low moisture contents limit the mobility and the supply of substrate to the soil microbes by diffusion through the soil solution. In wet soils, microbial activity is limited by the amounts of oxygen available for the decomposition process. The optimal environment for microbial activity is provided by a warm soil that is both moist and aerated. These conditions are met with intermediate moisture contents between those of dry and completely saturated soils. Soil moisture fluctuations, typical of arid and semiarid environments, are associated with pulses in the rates of decomposition and mineralization, with consequent pulses in the availability of soil mineral nutrients. (Wang et al. 2008).

However, when working with climate data summarized at a monthly or seasonal basis, quantifying the soil moisture conditions at a particular site is very difficult and soil moisture effects were not included in the statistical model.

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Crop yield and N utilization as influenced by C flux and stock components: a European multi-site experiment

Ferrise, R.[1 *], Antichi, D.[2], Thomsen, I.K.[3], Ventrella, D.[4], Mazzoncini, M.[2], Moriondo, M.[4], Olesen, J.E.[3], Bindi, M.[1], others

[1] Department of Agri-food Production and Environmental Sciences - University of Florence, P.le delle Cascine 18, 50144 Firenze

[2] Centre for Agro-Environmental Research "Enrico Avanzi" - University of Pisa, Via Vecchia di Marina 6, 56122 San Piero a Grado (Pisa), Italy

[3] Aarhus University

[4] Research Unit for Cropping Systems in Dry Environments of Agricultural Research Council, Bari, Italy

*Corresponding author: roberto.ferrise@unifi.it

Keywords

Soil organic carbon, Residue incorporation, Nitrogen fertilization, ^{15}N

1. Introduction

There is a large consensus that degradation of soils is one of the biggest threats to human development, as soils provide the most indispensable function of provision of food, feed and fiber for supporting the growing human population. At the same time they provide also a number of important agro-ecological services, as well as a range of regulating and supporting functions not least related to emissions and removals of greenhouse gases, and therefore to climate change mitigation (Smith, 2012). The majority of these soil functions are closely linked to the amount and quality of soil organic matter (SOM), which strongly affects many chemical, physical and biological properties of soils playing a significant role in determining also crop productivity (Allison, 1973).

The natural level of organic carbon in agricultural soils (the so called "*C stock*") depends on peculiar bioclimatic and pedogenetic conditions acting in the long term. On the other hand, the C stored in the soil can be also affected by crop management strategies. In particular, tillage and incorporation of organic materials (e.g. crop residues, organic fertilizers, manures, composts) can modify the rate and the quality of the transformation processes the soil organic matter is subjected to in the short-medium term (the so called "*C flows*") (Thomsen and Christensen, 2004; Lal and Kimble, 1997).

Intensive cultivation, low restitution of crop residues to the soil, simplified crop rotation and heavy use of pesticides are reported to have contributed to a global decrease of carbon historically stored in the soils, estimated by Lal (2004) in 42–78 Gt of carbon reversed to the atmosphere. This emphasizes the importance of an increased C sequestration in the soils for contributing to the reduction of global warming potential of the whole agricultural sector in the future. In intensive cropping systems, this might be possible through the adoption of conservation agriculture techniques, such as no-tillage and cover-cropping, which are well known to increase the levels of soil C, mainly through the protection of the SOM already present in the soil and the addition of C immobilized in cover crop biomass (Lal and Kimble, 1997). On the other hand, the use of conservative strategies, unless combined with high inputs of agrochemicals, is generally reported to decrease crop yields, due to the side effects of the replacement of inversion tillage (e.g. high weed pressure, low nutrient availability).

Furthermore, in many cases the adoption of strategies aiming at increasing soil C stock can interfere with natural processes sustaining crop productions. For instance, the constant incorporation into the soil of C rich materials, such as cereal straw or green manures, can substantially increase the immobilization rate of mineral nitrogen (N) in the microbial biomass, as a result of the unbalanced C:N ratio. This obviously reduces the availability of mineral N for the crops, and results at the end in low crop yields (Thomsen and Christensen, 2004). In these conditions, an increase in N fertilization levels is needed for maintaining crop yields at acceptable levels, meaning additional economic and environmental costs for farmers and the whole society and a loss of sustainability. Conversely, any change in the agricultural practices leading to significant regain in soil carbon should also, at least, preserve, or even increase crop productivity in a world where human population is constantly increasing. Therefore, a question still remains open on how to combine the goals of SOM conservation and crop productivity in intensive cropping systems. Nonetheless, the integrated effect of SOM on crop growth and yields is far to be elucidated because SOM derived effects are confounded with those of soil management (Branca et al., 2013). The application of the above mentioned concept of C flows and C stocks could make possible to isolate the effect of management from those of soil organic matter content, surmising that flows and stocks play separate and different roles in relation to soil properties, soil functions and crop productivity, and that soil management can be designed to optimize either flow-derived or stock-derived functions. According to this concept, the crop yield response to added organic material depends not only on the quantity and quality of the material, but also on the soil management that affects the temporal and spatial pattern of its decay (Christensen and Johnston, 1997).

In this paper, we adopt the C flows and C stock concept to study the interactive effects of crop residues management and N fertilization levels on grain yield and N utilization of winter wheat grown in cropping systems characterized by different levels of C stocks, as resulting from long-term application of SOM conservation strategies, and different pedo-climatic conditions. Our hypothesis to be tested is: irrespective of pedo-climatic conditions, the long-term accumulation of C into the soil (*C stock*) generates such high soil fertility level that the initial immobilization of soil mineral N due to the incorporation of wheat straw (*C flux*) would not produce significantly lower crop yields compared to soils managed without incorporation of crop residues.

2. Materials and methods

In this study, field trials aimed at exploring the crop response to N fertilization as influenced by soil organic carbon stock and flow were carried out. The flow/stock aspects were investigated in three long-term experiments: two located in Central and Southern Italy and one in Denmark. At each site, confined micro-plots were installed and high and low organic carbon flow was introduced under both high and low stock conditions. The crop response to fertilization covering up to 50% more than the standard N application at each site was explored.

2.1 Study sites

As summarized in table 1, the experimental sites differed in terms of climate, soil properties and crop management of the long term experiments (LTEs). In particular, differences in SOM content, within the same LTE, have been built up due to different crop residue management.

The LTE in Denmark, described in Thomsen and Christensen (2004), started in 1981 at Askov Experimental Station (55°28'N, 9°06'E). The climate is temperate with average annual temperature 7.6°C and cumulated precipitations 862 mm. According to the Soil Taxonomy System, the soil is classified as an Alfisol (typic Hapludalf) with a light sandy loam texture in the top 20 cm. The experiment consists of continuous spring barley sown in February-March in which 4 levels of residues incorporation (namely 0, 4, 8 and 12 t ha^{-1} yr^{-1}) were combined with addition or not of slurry and catch crop in a split-plot design. The soil is ploughed with mouldboard to 20 cm followed by conventional seedbed preparation. Plant density at sowing is 300-350 plants m^{-2}. The crop is fertilized with 100 kg N ha^{-1} supplied in spring during the seedbed preparation. Plots with no catch crop nor slurry treatment were used for our study. The low and high carbon stock condition used in this experiment has been built by either removing (low SOC stock) or incorporating the straw at a fixed rate of 8 t ha^{-1} (high SOC stock).

The LTE in Central Italy, has been conducted since 1993 at the Centre for Agro-Environmental Research (CIRAA) 'Enrico Avanzi' of the University of Pisa (43°40'N, 10°19'E) (Mazzoncini et al., 2011). The climate reflects the typical Mediterranean conditions with hot, dry summers and mild, wet winters. Mean annual temperature is 14.5°C. The coldest month is January with average monthly mean temperature 7°C. July and August are the hottest months with temperatures that may exceed 30°C for several days. Annual rainfall is on average 907 mm yr^{-1}, mainly concentrated in autumn with a secondary peak during spring. The soil is a Typic Xerofluvent with loam textural class in the top layer. The experiment was based on maize monoculture until 1998. Afterwards, a two-year crop rotation (maize-durum wheat) followed until 2005. Since then, a four-year crop rotation (durum wheat – maize –durum wheat - sunflower) has been practiced. The experiment compares in a factorial design three factors (i.e. tillage, N fertilization rate and soil cover type). A subset of treatments was extracted for the aim of this study. The soil tillage system is based on ploughing to 30 cm, followed by secondary tillage with disk harrow for weed control, fertilizer incorporation and seedbed preparation before fall or spring crop planting. Crop residues are always incorporated. Plots with high and low stock conditions have been respectively created by using or not cover crops. The cover crop selected in this study is a legume crop (*Vicia villosa* Roth) with a high potential of nitrogen accumulation. Sown in winter between wheat and maize or wheat and sunflower, the cover crop is killed at early flowering stage and ploughed into the soil before the spring crop.

The LTE in South Italy, described in Castrignanò et al. (), is located at the CRA-SCA Experimental Farm in Foggia (41°26'N, 15°30E) and began in 1990. The climate is typical Mediterranean with mean annual temperature 16.3°C and average annual rainfall 391 mm mostly concentrated in autumn and winter. During summer the rainfall shortage is coupled with hot temperatures that usually exceed 30°C. The soil has a clay-loam texture with an alluvial origin, classified by Soil Taxonomy-USDA as fine mesic, Typic Chromoxerert. The LTE is based on monoculture of durum wheat sown in autumn with a density of 450 seeds m^{-2}. Before sowing the soil is ploughed with mouldboard up to 40 cm depth, followed by disc-harrowing to 20 cm and by a rotary hoeing to 10 cm. During the principal ploughing phase, 100 kg ha^{-1} of P_2O_5 as mineral perphosphate are applied. The crop is dressed with 100 kg N ha^{-1} applied during spring at the beginning of the stem elongation. Crop residues are either burnt or incorporated to create low or high carbon stocks, respectively.

2.2 The experimental protocol

The experiment was conducted in the cropping season 2012-13. In each site, 2 carbon stock conditions (high and low stock), 2 carbon flow treatments (high and low flow) and 6 N fertilization levels were combined in a split-split-plot experimental design with 3 replicates (in total, 72 combinations).

After harvest 2012 general residues incorporation as well as burning of straw was suspended in a strip of the plots planned for the experiment. After ploughing, for each replicate, twelve cylinders were pressed into the soil in plots with low carbon stock (i.e. straw removal in Askov, no cover crop in Pisa and straw burning in Foggia) and another twelve cylinders into the soil in plots with high carbon stock (i.e. straw incorporation in Askov and Foggia and cover crop in Pisa). The cylinders were 30 cm in diameter and 30 cm in depth.

In order to create high flow conditions, wheat straw was incorporated in half of the cylinders in high carbon stock plots as well as in half of those in low carbon stock plots. The straw was chopped at circa 5 cm and portions to be incorporated in the top soil layer of the micro-plots were prepared. Each portion consisted of 57 g dry straw material for the Danish experiment and 36 g of dry straw material for both the Italian sites. Each straw portion corresponded to the typical straw incorporation rate at each site. That is, 8 t straw ha^{-1}.for the Danish LTE and 5 t straw ha^{-1} for the Italian sites. The other half of the cylinders were left without amendment to create the low flow conditions.

To allow for a crop response curve to fertilization, cylinders were supplied with six incremental rates of N from 0 kg N ha^{-1} to 50% more than normal rate at each site. For Denmark, N rates ranged from 0 kg N ha^{-1} to 250 kg N ha^{-1} with steps of 50 kg N ha^{-1}. In Italy, N was supplied, at intervals of 30 kg N ha^{-1}, up to the maximum rate of 150 kg N ha^{-1}. The N was distributed as ^{15}N-labelled fertilizer in spring 2013. Standard N was withheld from the cylinders and an area of 1 m around the cylinders.

Beside N fertilization, the management of the crop was left unchanged as the usual at each site. All sites were sown to wheat at a seeding rate typical of each location. In each micro-plot 24 seeds in Denmark (corresponding to 340 seeds m^{-2}), 32 seeds in Central Italy (450 seeds m^{-2}) and 35 seeds in South Italy (500 seeds m^{-2}) were sown. Phosphorous, potassium and other nutrients were supplied as in standard crop management. The crop was treated with herbicides and pesticides according to standards and the surrounding field.

Once during the season, leaves were sampled at a well defined growth stage (BBCH 45, end of booting) and were analysed for total N and ^{15}N. At maturity plants in each cylinder were cut 5 cm above soil surface. The plants from each cylinder were oven dried at 60 °C to constant weight. After threshing, the dry weight of grain and straw (including chaff) fractions as well as grain and straw total N and ^{15}N uptake were determined.

2.3 The statistical analysis

Data of grain, straw and total aboveground dry matter production of wheat grown in each site were analyzed separately with analysis of variance (ANOVA) for a split-split-plot design. C-stock was the main factor, C-flow the sub-plot factor and N fertilization level the sub-sub-plot factor. Using the graphic box-plot method, 7 and 9 outliers, respectively for Pisa and Foggia sites, were identified and excluded from the analysis. Due to the presence of outliers, the test used for comparing the differences between treatment means was the Tukey-Kramer test at $P<0.05$ (Gomez and Gomez, 1984). In case of significant effect of interactions between factors, the comparison among treatment means was performed with the LSD test. The statistical software used was CoStat Software (CoHort, 2002). Before the ANOVA, the Bartlett test was performed to test the homogeneity of error variances. For Askov site, the Bartlett test was significant for dry matter production of grain and total aboveground biomass. Therefore, these data were transformed in log10 (x) (Gomez and Gomez, 1984).

3. Results and discussion

Grain and straw dry mass at Askov (Denmark)

According to the statistical analysis, at Askov, wheat grain yield was modified by soil carbon stock (Cs) and N fertilization (Nf) for which significant interactions were also found. Final straw dry matter was only affected by carbon flow (Cf) and Nf main effects (Table 2).

Wheat grain yield increased as N fertilization increased, although at the highest N rates, a saturation effect was observed and grain yield tended to reach a plateau (Fig. 2). On average, at highest N inputs grain final dry mass was 3.6 times higher than in the unfertilized plots. Carbon stock positively influenced grain yield that was 34% higher in high Cs compared to low Cs plots. This effect may be ascribed to the higher N supply due to the mineralization of higher contents of soil organic matter in high Cs conditions (Thomsen and Christensen, 2004). Furthermore, it is likely to suppose that higher Cs may enhance the soil structure, thus affecting positively the accumulation of biomass. Similar conclusions were drawn by Schelde et al., in a study carried out within the SmartSOIL Project. The authors analyzed the effect of soil organic carbon on yield, in the same long term experiment used for conducting the trials in this study. They suggested that increasing level of soil organic carbon could be related to higher water holding capacity, thus leading to higher N utilization efficiencies by the crop.

Straw final dry mass was increased by increasing N rates (Fig. 2). Compared to unfertilized plots, straw final dry mass was 3.8 times higher at highest N doses. Similarly to grain yield, the effect of the highest N rates was not significant, thus indicating the reaching of a plateau. Although not significant (P=0.05) straw dry mass in high Cs was 21% higher than in low Cs. On average, Cf main effect reduced the straw final dry mass by ca. 10% in the high Cf plots. This latter effect is likely due to the N immobilization consequent to the incorporation of organic material with a high C:N ratio such as the wheat straw used in this experiment.

Grain and straw dry mass at Pisa (Central Italy)

The statistical analysis revealed that both grain and straw final dry matter were significantly affected by C flow and N rates main effects, while no difference were observed in response to the different soil C stocks. None of the interactions between the main factors was significant (Table 2 and Fig. 2).

Grain and straw final dry mass increased in response to increasing rates of N fertilization, without showing saturation effects at higher N fertilizer levels. Averaged over the other treatments and compared to the unfertilized plots, grain yield as well as straw final dry mass were doubled at highest N fertilization rates.

The incorporation of wheat straw into the soil significantly reduced crop performance, leading to lower final dry mass of both grain and straw. The main reason behind this observation may be the higher immobilization of soil mineral N under high than low C flow, due to the huge amount of organic C added to the soil with the incorporation of the straw (higher C:N ratio under high than low flow).

C stock did not affect crop performance at all. This might have been likely due to the very low differences in C stock between the two treatments (about 1.6 in the low stock vs 1.9 in the high stock g S.O.M. 100 g^{-1}). The effect of the legume cover crop on the availability of N for the wheat was not revealed by dry matter production data. This might have been due to the fact that the cover crop was incorporated into the soil in spring 2012, thus the N sparing effect of the legume might have been not relevant for the second next cash crop (i.e. wheat grown in 2012/13 after sunflower). Furthermore, the huge amount of rainfall occurred in winter 2012/13 (about 1500 mm) might have leached most of the residual N present in the topsoil.

Grain and biomass at Foggia (South Italy)

At Foggia, the C flow significantly affected both grain yield and straw dry mass. On the contrary, there were no statistically significant effects of C stock, nor of the N fertilization on the final accumulation of grain and straw dry mass.

Also in this case, the incorporation of the residues caused the final accumulation of grain and straw dry mass to be reduced. Compared to low flow conditions, at high Cf grain yield was reduced by 23.4% and straw by 21.5%. This response reinforces the idea of an immobilization of the soil N due to the incorporation of material with high C:N ratio.

As in Pisa, the lack of response to different C stocks may be ascribed to the very low difference between high and low C stock plots (1.3 g S.O.C 100 g^{-1} in low stock plots vs. 1.5 g S.O.C 100 g^{-1} in high stock plots). It is likely to suppose that, compared to low Cs, high Cs neither supplied the crop with significantly greater availabilities of N from mineralization nor modified the soil structure so as to enhance the soil water holding capacity.

Although not statistically significant, both grain and straw final dry mass tended to increase in response to higher N inputs. This response may be ascribed to the late sowing date (22nd January), and to the reduced precipitation that cause water to be the limiting factor, thus not allowing the crop to exploit the potential of higher N fertilizations.

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Tables

Table 1: Characteristics of the experimental sites

Site	**Askov (Denmark)**	**Pisa (Central Italy)**	**Foggia (South Italy)**
Location	55°28’N, 09°06’E	43°40´ N, 10°19´ E	41°26' N, 15°30' E
Avg. annual temp. (°C)	7.6	14.5	16.3
Avg. precipitation (mm)	862	907	391
Soil characteristics (0-20 cm):			
Soil type	Alfisol (Typic Hapludalf)	Typic Xerofluvents	Typic Chromoxerert
Clay, <2 μm (mg g^{-1})	130	155	494
Silt, 2<20 μm (mg g^{-1})	110	402	311
Sand, >20 μm (mg g^{-1})	738	443	195
LTE characteristics:			
Experiment initiated	1981	1993	1990
Crops in rotation	Continuous spring barley	Continuous maize (from 1993 to 1998) Maize-Durum wheat (from 1998 to 2005) Maize-Durum wheat-Sunflower-Durum wheat (since 2006)	Continuous durum wheat
Experimental treatments selected for SmartSOIL new exps.			
low carbon stock	straw removal	no cover crop	straw burning
high carbon stock	straw incorporation (8 t ha^{-1} yr^{-1})	high N supply legume cover crop	straw incorporation

Table 2: Mean effect of C stock (Cs), C flow (Cf), N fertilization (Nf), and their interactions on dry matter production (t ha^{-1}) of grain, residues and total aboveground biomass of wheat sampled at harvest maturity.

Effect	Askov			Pisa			Foggia		
	Grain yield (t ha^{-1})	Residues dry matter (t ha^{-1})	Total dry matter (t ha^{-1})	Grain yield (t ha^{-1})	Residues dry matter (t ha^{-1})	Total dry matter (t ha^{-1})	Grain yield (t ha^{-1})	Residues dry matter (t ha^{-1})	Total dry matter (t ha^{-1})
C stock[1]	**	ns (.05)	*	ns	ns	ns	ns	ns	ns
C flow[2]	ns (.06)	*	*	***	***	***	***	***	***
N level[3]	***	***	***	***	***	***	ns (.10)	ns (.08)	ns (.08)
Cs x Cf	ns	ns	ns	ns	ns	ns	**	ns (.07)	**
Cs x Nf	*	ns	*	ns	ns	ns	ns	ns	ns
Cf x Nf	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs x Cf x Nf	ns	ns	ns	ns	ns	ns	ns	ns	ns
C stock[1]									
High	6.33 a	4.93	11.26 a	3.66	5.31	8.97	2.74	4.30	7.04
Low	4.73 b	4.07	8.80 b	3.71	5.17	8.88	2.84	4.35	7.19
C flow[2]									
High	5.34	4.27 b	9.61 b	3.34 b	4.73 b	8.07 b	2.42 b	3.80 b	6.22 b
Low	5.72	4.72 a	10.44 a	4.05 a	5.76 a	9.81 a	3.16 a	4.84 a	8.00 a
N level[3]									
N1	1.76 e	1.57 e	3.33 e	2.20 e	3.36 e	5.56 e	2.30	3.63	5.93
N2	3.51 d	3.00 d	6.51 d	2.87 de	4.28 de	7.15 de	2.48	4.02	6.50
N3	5.01 c	4.20 c	9.21 c	3.42 cd	4.83 cd	8.25 cd	2.74	4.32	7.06
N4	7.02 b	5.67 b	12.69 b	3.93 bc	5.56 bc	9.49 bc	2.89	4.48	7.37
N5	7.76 ab	6.13 ab	13.89 a	4.38 ab	6.16 ab	10.54 ab	3.00	4.48	7.48
N6	8.11 a	6.42 a	14.53 a	4.96 a	6.76 a	11.72 a	3.13	4.75	7.88

In the same column, treatment means followed by different letter are significantly different at $P < 0.05$ (Tukey Kramer's test).

[1] High and Low are, respectively, treatments with highest and lowest soil organic carbon content, as result of the long-term management

[2] High and Low are, respectively, treatments with wheat straw incorporated or not into the soil before the sowing date of winter wheat

Figure captions

Figure 1: Response of grain (upper panel) and straw (bottom panel) final dry mass to increasing N fertilization rates at Askov (Denmark). Data are average ± 1 s.e. for n=3 independent replicates.

Figure 2: Response of grain (upper panel) and straw (bottom panel) final dry mass to increasing N fertilization rates at Pisa (Central Italy). Data are average ± 1 s.e. for n=3 independent replicates.

Figure 3: Response of grain (upper panel) and straw (bottom panel) final dry mass to increasing N fertilization rates at Foggia (South Italy). Data are average ± 1 s.e. for n=3 independent replicates.

Figure 1

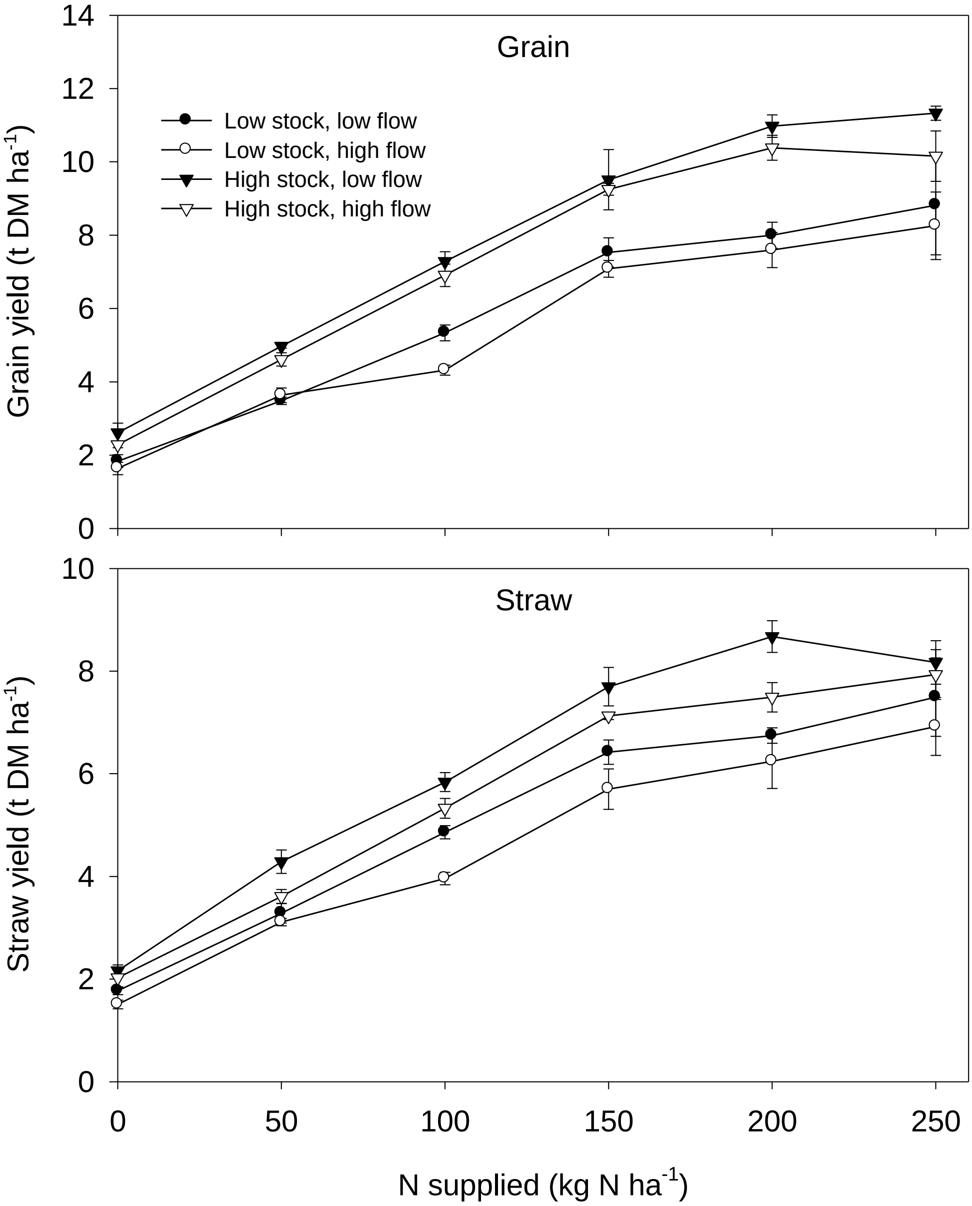

Grain
Low stock, low flow
Low stock, high flow
High stock, low flow
High stock, high flow
Grain yield (t DM ha^{-1})
0
2
4
6
8
10
12
14
Straw
Straw yield (t DM ha^{-1})
0
2
4
6
8
10
0
50
100
150
200
250
N supplied (kg N ha^{-1})

Figure 2

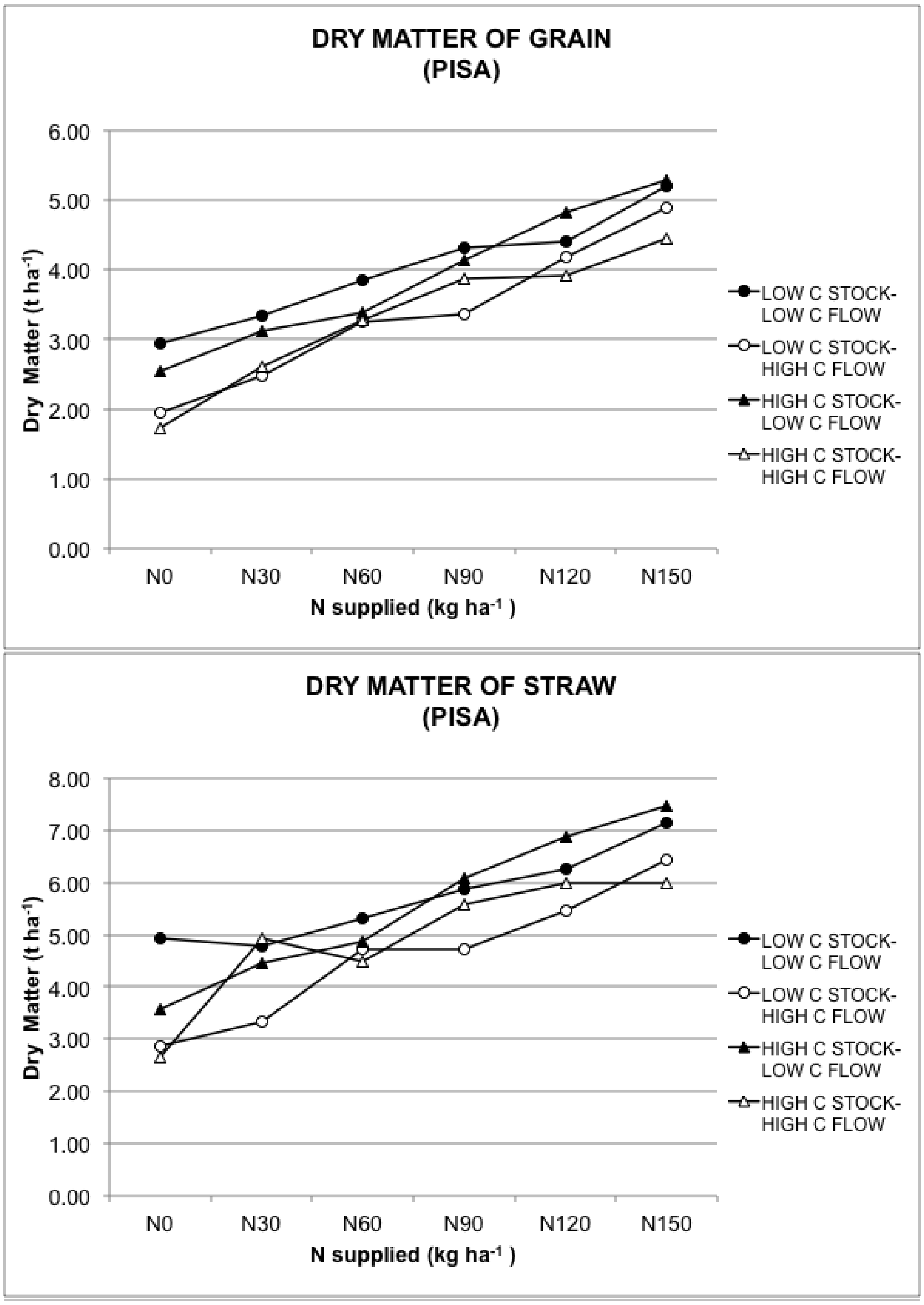
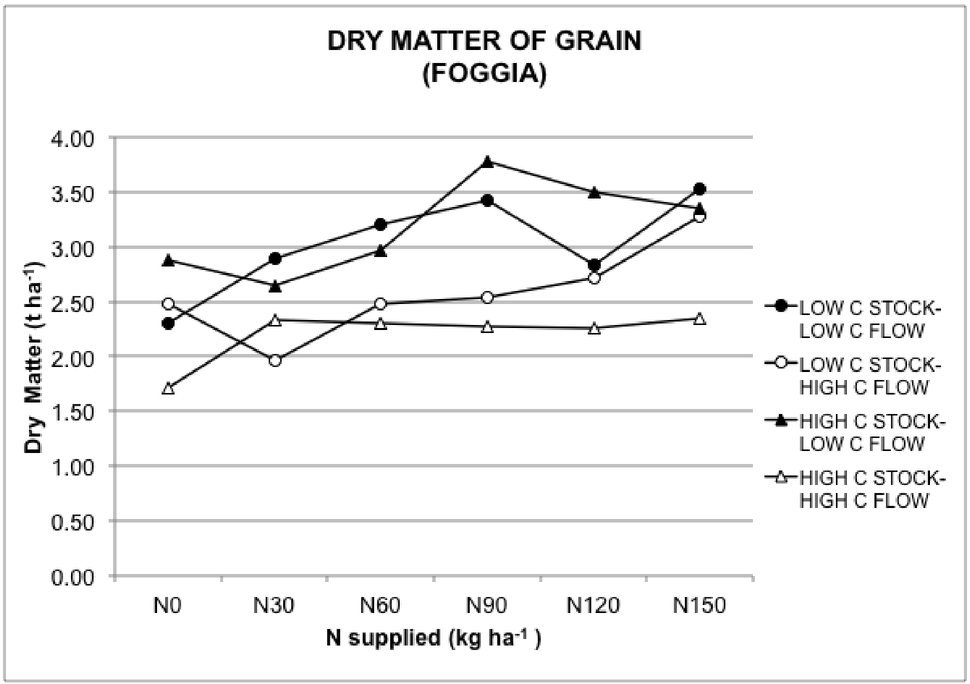
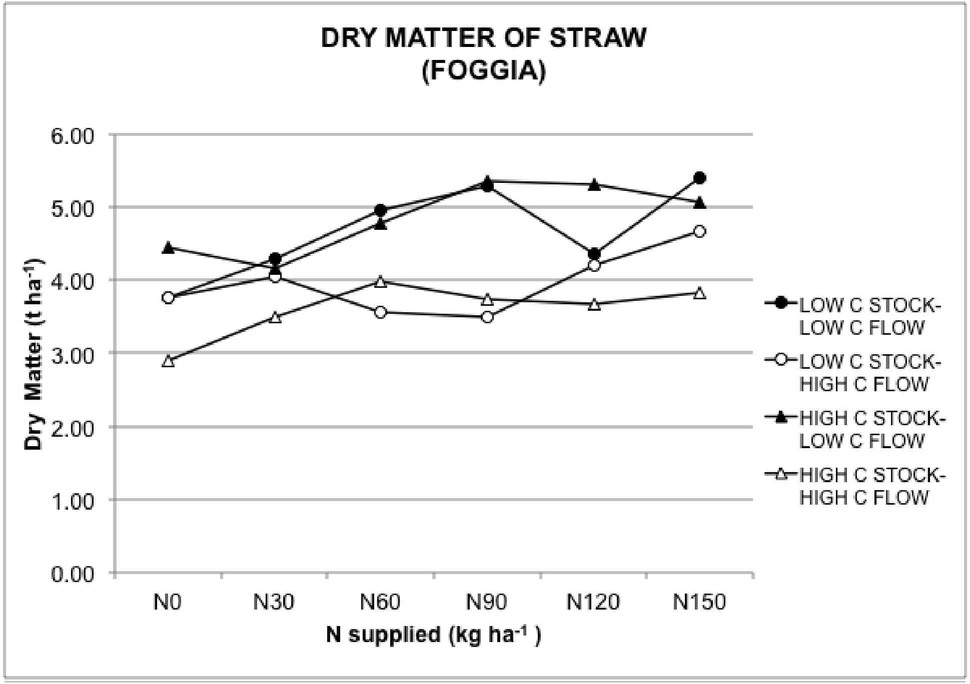

DRY MATTER OF GRAIN
(PISA)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Dry Matter (t ha^{-1})
N0
N30
N60
N90
N120
N150
N supplied (kg ha^{-1})
LOW C STOCK-LOW C FLOW
LOW C STOCK-HIGH C FLOW
HIGH C STOCK-LOW C FLOW
HIGH C STOCK-HIGH C FLOW
DRY MATTER OF STRAW
(PISA)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Dry Matter (t ha^{-1})
N0
N30
N60
N90
N120
N150
N supplied (kg ha^{-1})
LOW C STOCK-LOW C FLOW
LOW C STOCK-HIGH C FLOW
HIGH C STOCK-LOW C FLOW
HIGH C STOCK-HIGH C FLOW

Figure 3


DRY MATTER OF GRAIN
(FOGGIA)
Dry Matter (t ha^{-1})
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
N0
N30
N60
N90
N120
N150
N supplied (kg ha^{-1})
LOW C STOCK-LOW C FLOW
LOW C STOCK-HIGH C FLOW
HIGH C STOCK-LOW C FLOW
HIGH C STOCK-HIGH C FLOW


DRY MATTER OF STRAW
(FOGGIA)
Dry Matter (t ha^{-1})
6.00
5.00
4.00
3.00
2.00
1.00
0.00
N0
N30
N60
N90
N120
N150
N supplied (kg ha^{-1})
LOW C STOCK-LOW C FLOW
LOW C STOCK-HIGH C FLOW
HIGH C STOCK-LOW C FLOW
HIGH C STOCK-HIGH C FLOW

The following draft is intended for a stand-alone paper or, more likely, for merging with a similar study in a paper on soil health indicators.

Arbuscular mycorrhiza fungi as soil health indicator

Sabine Ravnskov, Agroecology, Aarhus University, Denmark

Introduction

A healthy agricultural soil can be defined as a soil without or with a low amount of functional plant pathogens. Soil health can, however, be difficult to evaluate based on presence of plant pathogens. Firstly, because biomass of soil borne plant pathogens seldom is correlated with the risk of plant disease in the crop, secondly as most pathogens are adapted to one or few host plants, and with crop rotation soil health will vary depending on the specific crop in the field. As soil health is related to the single crop, a soil healthy for one crop is not necessary healthy for another. However, a healthy soil can also be characterized by containing general plant beneficial microorganisms suppressing the soil borne plant pathogens.

Arbuscular mycorrhizal fungi (AMF) are naturally occurring in soils all over the world. Around 200 species are described so far, and they have their own fungal phylum, *Glomeromycota* (Schüssler et al., 2001). AM fungi are obligate biotrophic fungi in plant roots. Evolutionary, they are the oldest known fungi, and have co-evolved with plants for more than 460 million years (Redecker et al., 2000). Approximately 80% of all investigated plants form mycorrhiza under natural growth conditions (Smith and Read, 2008). Functionally, the symbiosis between AM fungi and plants are characterized by reciprocal exchange of nutrients as the fungus obtains carbon from the plant and the plant receives inorganic nutrients and water from the fungus.

AMF provide an ecosystem service for most crops by their strong impact on plant nutrient uptake, growth, and drought tolerance (Gianinazzi et al. 2010; Smith and Smith 2011), and by increasing plant tolerance against a range of soil borne pathogens in different crops (Whipps, 2004). Moreover, AM fungal colonization of roots has been shown to influence the interaction between insect herbivores and their natural enemies, and thereby biological pest control (Hartley and Gange 2009). The key role of AMF in plant health by increasing plant tolerance against pathogens were demonstrated by Yu et al. (2012), and followed up by Xu et al. (2012), who identified AMF as soil health indicator. AM fungi has also been shown to increase plant tolerance against abiotic stress in soil e.g. drought (Jayne & Quigley, 2014), high salt concentrations (Estrada et al., 2013) and nutrient limitation, especially of phosphorous (Smith & Smith, 2011). This higher plant tolerance against abiotic stress can be explained by the capacity of the AM fungi to take up nutrients and water inaccessible for the plant and to transport it to the plant *via* the root external mycelium.

AMF strongly influence carbon flow in the agricultural system both by influencing photosynthetic rate of the plants, but also by gaining 4-20 % of the carbon fixed by plants via photosynthesis, and thereby considerably influence carbon flux to the soil (Jakobsen and Rosendahl, 1990), as a major part of the AMF carbon is fixed in AM fungal mycelium and spores in the soil, and contribute significantly to the microbiological pool of carbon in the soil (Olsson 1999). On the other hand, quality and quantity of soil organic matter has shown to influence on biomass of AMF in soil and on AM functioning (Ravnskov et al. 1999 Albertsen et al. 2006)

Aim and objective

The aim was to study the possible link between carbon sequestration and soil health.
The objective was to study the influence of incorporation of organic matter, mechanical soil management and crop rotation on soil health as measured by presence of a soil health indicator organism; arbuscular mycorrhizal fungi (AMF).

Materials and methods

Soil and wheat roots were sampled in long term experiments in order to evaluate influence of different management strategies and of carbon sequestration on AMF inoculum potential of the soil as an expression of soil health. AMF in roots and soil was measured by determination of the biomarker fatty acid for AMF; 16:1ω5 (Olsson et al 1999).Both the neutral lipid fatty acid (NLFA) and the phosphor lipid fatty acid (PLFA) of 16:1ω5 were quantified as an expression of storage and membrane bound lipids of AMF, respectively. As the membrane bound PLFA 16:1ω5 also can be found in bacteria in soil, only NLFA analysis was used for evaluation of AMF inoculum potential of soils.

Experimental sites

The experimental sites are identical with the sites described in Ferrise et al. (2014). The main experimental site was at Askov, Denmark, where a full factorial experiment with the following three main factors was employed ; 1. Incorporation of straw into the soil (four levels; 0, 4, 8 or 12 t ha^{-1}); 2. Time of soil ploughing (two levels; autumn or spring) and 3. Pre-crop (three levels; none, rye grass or rye grass/clover). In the field, each treatment had three replicate plots placed in a randomized block design.
Moreover, soil and roots were sampled in two selected treatments in the two Italian experiments, in Foggia and in Pisa, respectively. From the Foggia Exp., the treatment with burning of stubble and wheat straw (T1), and the treatment with incorporation of stubble and wheat straw into the soil (T2) was included. From the experiment in Pisa, the weedy control treatment (C) and the Vicia villosa (Vv) treatment were included.

Soil and root sampling

First soil sampling was in the autumn 2012, just before sowing in the field. Second soil sampling and root sampling was in June 2013 just before harvest, in growth phase 75-85 on the BBCH scale (maturing).
Ten soil cores (diameter 20 mm) in the upper 10 cm soil were taken randomly in each plot. Subsamples from each plot were pooled and carefully mixed in order to have one representative sample from each plot. Roots were sampled from five replicate plants randomly selected in the middle area of each plot. Soil was washed of the roots using tap water; the five replicate root systems from each plot were pooled before further analysis. Both soil and root samples were transported at cooled conditions to the lab, lyophilized, milled under liquid nitrogen and stored in the freezer (-20°C) until the analyses of biomarker fatty acids.

Analyses of biomarker fatty acids in soil and roots

Fatty acids profiles were analyzed by using a HP5890 gas chromatograph fitted with a 25 m fused silica column (HP part No. 19091B-102) with hydrogen as carrier gas, and with a HP chem station. Analytical details of the technique can be found in Sasser (1990). The fractionation of total soil lipids consisted in soil extraction followed by lipid fractionation by solid phase extraction to obtain the neutral-, glyco- and phospho-lipids fractions. In this work,

the NLFA fraction of soils and the PLFA and NLFA fractions of roots were analyzed. Briefly, 3 g of soil/roots were extracted with a citrate buffer-chloroform-methanol mixture, the organic layer was fractionated through a solid phase extraction column filled with silica, and neutral, glyco and phospho-lipids were eluted with chloroform, acetone and methanol, respectively. The fractions were then exposed to a mild alkaline methanolysis, extracted with hexane, amended with 33.75 µg of the standard fatty acid 19:0 (not found in environmental samples), evaporated under N_2 stream, and resuspended in 100 µl of hexane. Finally, 2 µl of the lipid extract from each lipid fractions were injected in the column of the gas chromatograph. The analysis was performed with an Agilent 6890 plus gas chromatograph, with a flame ionization detector, H_2 as carrier gas and an Ultra 2.5% phenyl methyl siloxane capillary column (25m x 200µm x 0.33 µm). The initial oven temperature of 170^{o}C was increased to 260^{o}c at 5^{o}C min^{-1}, followed by another increase to 310^{o}C at 40^{o}C min^{-1}. Each fatty acid concentration in the sample was estimated in relation to the internal standard *19:0*.
Biomarker fatty acids were identified using the Sherlock version 6.0 (MIDI inc) microbial identification protocol (Microbial ID, Newark, DE, USA). Specific biomarkers of taxonomic groups of microorganisms were analyzed. They were the followings: 16:1ω5c for AMF (Olsson 1999); 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso and 17:0 anteiso for Gram positive bacteria; 17:0 cyclo (Welch et al 2010) and 19:0 cyclo for Gram negative bacteria (Welch et al 2010); 16:0 10Me and 17:0 10Me for actinomycetes (Ratledge and Wilkinson 1988); 18:2ω6,9c, 18:1ω7 and 18:1ω9 for fungi (Frostegård and Bååth 1996) and 20:4 for protozoans.

Results and conclusions

Overall, the work showed a positive correlation between amount of incorporated straw and AMF inoculum potential of the soil revealing that straw incorporation increased both soil health and soil microbial carbon pool. This result could not be clearly confirmed by the experiment in Foggia, Italy, as soils with burning of straw had initially before sowing in 2012 a significant higher AMF inoculum potential than soil in treatments with incorporation of straw. However, during the Foggia experiment, AMF inoculum potential of soils with straw incorporation increased markedly more than in soils with burning indicating an overall up regulating effect of incorporation of straw as in the Askov experiment. The pre-crop significantly influenced AMF colonization of wheat plants in the Askov experiment; colonization of wheat with rye grass and clover was higher than in wheat with rye grass only as pre-crop. This was, however, not reflected in the AMF inoculum potential of the soil at harvest. AMF inoculum potential in soil in the Pisa experiment was not influenced by weedy control as compared in soil with Vicia villosa as pre-crop.

Table 1. Relative amount of the signature fatty acid for arbuscular mycorrhizal fungi 16:1ω5 in soil and wheat roots measured before and after different management practice in the field. Relative values in percent of total amount of fatty acids. NLFA=Neutral Lipid Fatty Acid; PLFA=Phospho Lipid Fatty Acid. Different letters after values in same row indicate significant differences as tested by post ANOVA multiple range test ($\text{LSD}_{0.05}$)

Time of ploughing	Pre-crop	Tons of straw incorporation	NLFA soil 2012	NLFA soil 2013	PLFA soil 2013	NLFA roots	PLFA roots
Autumn	None	0	3.84 bc	3.80 a	0.90 a	2.76 a-c	0.48 e
Autumn	None	4	3.82 bc	4.67 a-c	1.16 a-g	1.47 ab	0.33 a-e
Autumn	None	8	3.83 bc	6.61 c-f	1.40 g-h	3.17 a-c	0.44 b-e
Autumn	None	12	6.09 d	7.25 e	1.53 h	1,70 a-c	0.40 a-e
Spring	None	0	3.86 c	5.47 a-f	0.92 ab	2.40 a-c	nd
Spring	None	4	2.09 a	3.87 a	1.07 a-f	3.53 c	0.38 a-e
Spring	None	8	3.15 a-c	5.54 a-f	1.16 a-g	2.23 a-c	0.36 a-e
Spring	None	12	3.69 a-c	7.11 ef	1.27 c-h	1.41 a	0.24 a
Autumn	Rye grass	0	4.01 a-d	7.55 e	0.92 ab	2.86 a-c	0.38 a-e
Autumn	Rye grass	4	3.70 a-c	6.78 c-f	1.32 d-h	2.20 a-c	0.37 a-e
Autumn	Rye grass	8	3.35 a-c	6.92 d-f	1.42 gh	2.06 a-c	0.40 a-e
Autumn	Rye grass	12	3.69 a-c	6.39 c-f	1.33 e-h	2.18 a-c	0.45 c-e
Spring	Rye grass	0	2.13 ab	5.92 a-f	0.97 a-d	1.83 a-c	0.38 a-e
Spring	Rye grass	4	2.99 a-c	5.71 a-f	1.17 a-g	1.85 a-c	0.25 a
Spring	Rye grass	8	2.74 a-c	5.92 a-f	1.31 d-h	1.84 a-c	0.40 b-e
Spring	Rye grass	12	3.71 a-c	6.28 c-f	1.42 gh	2.99 a-c	0.48 de
Autumn	Rye grass and clover	0	3.67 a-c	4.82 a-d	1.04 a-e	2.10 a-c	0.30 ab
Autumn	Rye grass and clover	4	2.91 a-c	6.00 a-f	1.25 b-h	1.51 ab	0.35 a-e
Autumn	Rye grass and clover	8	3.60 a-c	6.00 a-f	1.23 a-h	1.48 ab	0.34 a-d
Autumn	Rye grass and clover	12	3.19 a-c	7.26 e	1.38 e-h	1.58 ab	0.31 a-c
Spring	Rye grass and clover	0	3.72 a-c	4.02 ab	0.96 a-c	1.63 a-c	0.29 a-d
Spring	Rye grass and clover	4	4.19 cd	5.12 a-f	1.34 e-h	3.31 bc	0.31 a-e
Spring	Rye grass and clover	8	3.54 a-c	6.13 b-f	1.34 e-h	1.33 a	0.35 a-e
Spring	Rye grass and clover	12	4.11 c	5.03 a-e	1.24 a-h	2.05 a-c	0.34 a-e
				Analysis of variance P values			
Time of ploughing			0.08	0.06	0.227	0.940	0.182
Pre-crop			0.290	0.09	0.581	0.116	*
Straw incorporation			0.17	**	***	0.789	0.727

*=P≤0.05;**=P≤0.01;***=P≤0.001 nd=not detected

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Crop yield responses to input intensity under varying soil carbon stocks and flows

K. Schelde, I. Thomsen, E.M. Hansen, M. Bindi, R. Ferrise, B. Ghaley, P. Kuikman, P. Smith, J.E. Olesen

Introduction

Soil organic matter (SOM) contributes to the nutrient supply to crops by enhancing the N cycling in the soil (Christensen and Johnson 1997, Luxhøi et al. 2007). SOC may also contribute to enhance soil fertility and soil productivity in other ways e.g. by improving soil structure and hence plant water availability or root development in search for micronutrients (Johnston et al. 2009, Diacono and Montemurro 2010). Previous meta-analysis studies, e.g. Pan et al. (2009), have reported positive correlations between SOM and soil or crop productivity. However, generally it is difficult to show and establish cause-effect relations: did soil C affect crop yields or did repeated high yields and associated inputs of crop residues affect the soil C status? Likewise, it is generally difficult to deduce whether long-term inputs of carbon to soils (leading to enhanced soil C stocks), or recent carbon inputs in the form of organic manure or crop residues (contributing to enhanced soil C and N flows via mineralisation processes) contribute the most to potential beneficial effects of SOC on crop yields. One reason is that 'old' and recent organic inputs are confounded in many experimental field studies (e.g., Silgram and Chambers 2002).

The aim of this analysis was to segregate, from the existing literature, effects of soil carbon stock and flows on crop productivity (crop yield in terms of dry matter yields and nitrogen uptake). We sought to explore how varying C stocks and flows affect crop yield responses to varying inputs; in this case inputs of fertilizer N. To assess the main effects of soil carbon stock, we identified two published studies with varying soil C stocks influenced by previous management consisting of incorporated organic matter and the use of catch crops. The two studies determined yield-to-N response curves over 3-4 growing seasons subsequent to the establishment of varying soil C stock and labile C pool levels. The experimental approach enabled us to distinguish possible effects of SOC on crop productivity that are beyond effects of indirect N supply to the crop.

Materials and methods

We analysed two datasets whose main results and trends were previously reported in Hansen et al. (2000a; dataset JYN) and in Thomsen and Christensen (2004; dataset ASK).

Dataset JYN

Hansen et al. (2000a) grew spring wheat on a coarse sandy soil (site JYN) during 1993-1996 in four treatments where the use of catch crops (CC) was either introduced, continued or discontinued compared to the period prior to 1993 (Table 1). The four treatments were A) CC introduced in 1993, B) long-term CC, C) CC until 1993, and D) never CC. In the two treatments (B and C) where catch crops were grown before 1993, this had been practiced since 1968. We term the period 1968-1992 the 'accumulation phase'. The CC was a perennial ryegrass under-sown each spring and incorporated by ploughing in late autumn. In the build-up phase from 1987 to 1992 the treatments were fertilized at low (60 kg N/ha/y) or high (120 kg N/ha/y) N rates. From 1968 to 1986 the N rates were a little higher (70 and 150 kg N/ha). Further, the treatments A and B were ploughed in autumn since 1987 (before that rotovated or direct drilled) and treatment C and D were ploughed in spring since 1968 (Hansen and

Djurhuus, 1997). In the 'test phase' (1993-96) four N levels (0, 60, 90 or 120 kg N/ha/y) were superimposed on the treatments A-D (Table 1).

Table 1. Overview of treatments in the accumulation and test phases of the JYN dataset. Darker cells indicate presence of catch crops. Accumulation phase was 1968-1992, test phase was 1993-1996.

	A		B		C		D	
Accumulation phase	AP		AP		SP		SP	
	No CC		CC since 1968		CC since 1968		No CC	
	High	Low	High	Low	High	Low	High	Low
Test phase	CC		CC		No CC		No CC	
	NRC	NRC	NRC	NRC	NRC	NRC	NRC	NRC

A, B, C, D: Experimental treatment identifiers
AP, SP: Autumn ploughed, spring ploughed
CC: Catch crops grown
High, Low: N fertilizer input levels in the accumulation phase, 120 or 60 kg N/ha
NRC: Crop yield N response curve established; 0, 60, 90 or 120 kg N/ha applied

Hansen et al. (2000a) showed that the different treatments led to different yields in plots with or without a previous history of CC incorporation. Higher yields, at a given N input rate, were obtained for plots with a CC history compared to plots without a CC history (Fig 2a in Hansen et al. 2000a). Yields tended to equalize between treatments after three years of the experimental test phase (Fig 3 in Hansen et al. 2000a). Moreover, Hansen et al. (2000b, table 4) showed that growing CC in the accumulation phase (treatment C) led to higher soil C levels compared to plots with no previous CC-history (treatments A and D). However, soil carbon content for treatment B with a CC history was not found to be higher than for treatment D where CC never was neither grown in the accumulation nor test periods. Average SOC contents ranged from 1.52% (treatment A) to 1.88% (treatment C).

Dataset ASK

Thomsen and Christensen (2004) established varying levels of soil C content on a light sandy loam (site ASK) by long term (accumulation phase of 18 years) incorporation of different rates of straw (0-12 t/ha/y), and catch crop (with or without). During the accumulation phase, grain crops grown in the treatments were fertilized with mineral fertilizer (100 kg N/ha/y). During a subsequent test phase (3 years: 2000, 2001, 2002), winter wheat was fertilized with mineral fertilizer at four varying rates (0, 60, 120, or 180 kg N/ha/y) while the other treatments were discontinued. See Table 2.

Thomsen and Christensen (2004) showed that grain yields were significantly increased in treatments with high (8 or 12 t) straw incorporation compared to straw removal. The effect was most prominent during the first year of the experimental test phase (Fig. 2 in Thomsen and Christensen, 2004).

Table 2. Overview of treatments in the accumulation and test phases of the ASK dataset. Darker cells indicate presence of catch crops. Yellow cells indicate high input of straw. The accumulation phase was 1981-1999, test phase was 2000-2002.

Accumulation phase	CC				No CC			
	0	4	8	12	0	4	8	12
Test phase	No CC, no straw				No CC, no straw			
	NRC	NRC	NRC	NRC	NRC	NRC	NRC	NRC

CC: Catch crops grown
0, 4, 8, 12: Straw input levels in the accumulation phase, 0, 4, 8, or 12 t/ha
NRC: Crop yield N Response Curve established; 0, 60, 120, or 180 kg N/ha applied

The straw treatments increased SOC in the ASK soil with increasing amounts of straw added (Thomsen and Christensen 2004, table 1). In 1999, the year prior to the test phase, SOC as a function of straw input rates in the build-up phase was 1.27, 1.42, 1.54 and 1.65 % for input rates of 0, 4, 8, and 12 t ha^{-1} y^{-1}, respectively. In 2002, after the test phase, SOC levels were still significant and varied from 1.24 to 1.55% for the same range of straw inputs. Further, Thomsen and Christensen (2004) found that a third treatment with pig slurry applied to the ryegrass catch crop affected neither soil C and N concentrations nor crop yields. Therefore the slurry treatments were not considered for the present analysis and the data were pooled with the remaining treatments.

Results and discussion

We analysed Yield- N uptake relationships in the JYN dataset. Figure 1 is based on data in individual research plots. Each year (1993-1996) there seemed to be a smooth relationship (Fig 2 left) between grain yield and grain N uptake. This development was regardless of CC history.

The grain DM yield to N-uptake ratio is equivalent to the crop physiological nitrogen utilization efficiency (NUE) (Giambalvo et al. 2010). The levelling out of the curves in Fig. 2 at high N uptake rates shows how higher N uptake is gradually traded into a lower NUE. Each year (1993-1996) there was a negative relationship between NUE and N uptake (Fig. 2 right). There was however also a tendency towards stagnant or lower NUE at low N uptake rates (<30 kg N/ha) after 1993, the initial year. There were similar types of relationships between NUE and N uptake regardless of CC history.

Likewise we analysed Yield – N uptake relationships in the ASK dataset. Figures 2-4 are based on observations in individual research plots. Compared to JYN (Fig. 1), there was a less smooth grain yield versus N-uptake relationship (Fig 2). For individual high N fertilization levels (120N, 180N) there seemed to be steeper relationships compared to a general model stabilizing at high N uptake rates. Higher yields, for a given N input, were obtained at sites with a high straw input compared to sites with a low input, as also pointed out by Thomsen and Christensen (2004). The relation between yield and N uptake was not clearly influenced by catch crop history.
Considering physiological NUE in the harvested grain, there was a general decrease in NUE for increasing N uptake (Fig 3). This was equivalent to the general trend seen also for the JYN site (Fig. 1).
There was however a contrasting trend in the ASK data, indicated by black trend lines in Figure

3. The contrasting trend was most prominent during 2000. In these cases, increasing N uptake resulted in weakly increasing physiological NUE. This effect might be related to other factors than N-nutrition, since we would expect N made available in excess of mineral N applied in early spring (such as N released from mineralisation of organic matter during the growing season) to lead to a higher build-up of protein in the grains and thus a higher N%, equivalent to a lower NUE.

The contrasting effect was quantified by calculating the difference between actual NUE and a general linear model for NUE as a function of N uptake. This model accounted for the general linear decrease in NUE with N uptake at the ASK site for N input levels at 60 kg N/ha and above. Individual models were calculated for each year. We named the computed differences NUE residuals. The higher the NUE residual, the better the NUE obtained for a given N uptake rate. Hence positive residuals would be advantageous in terms of high NUE.

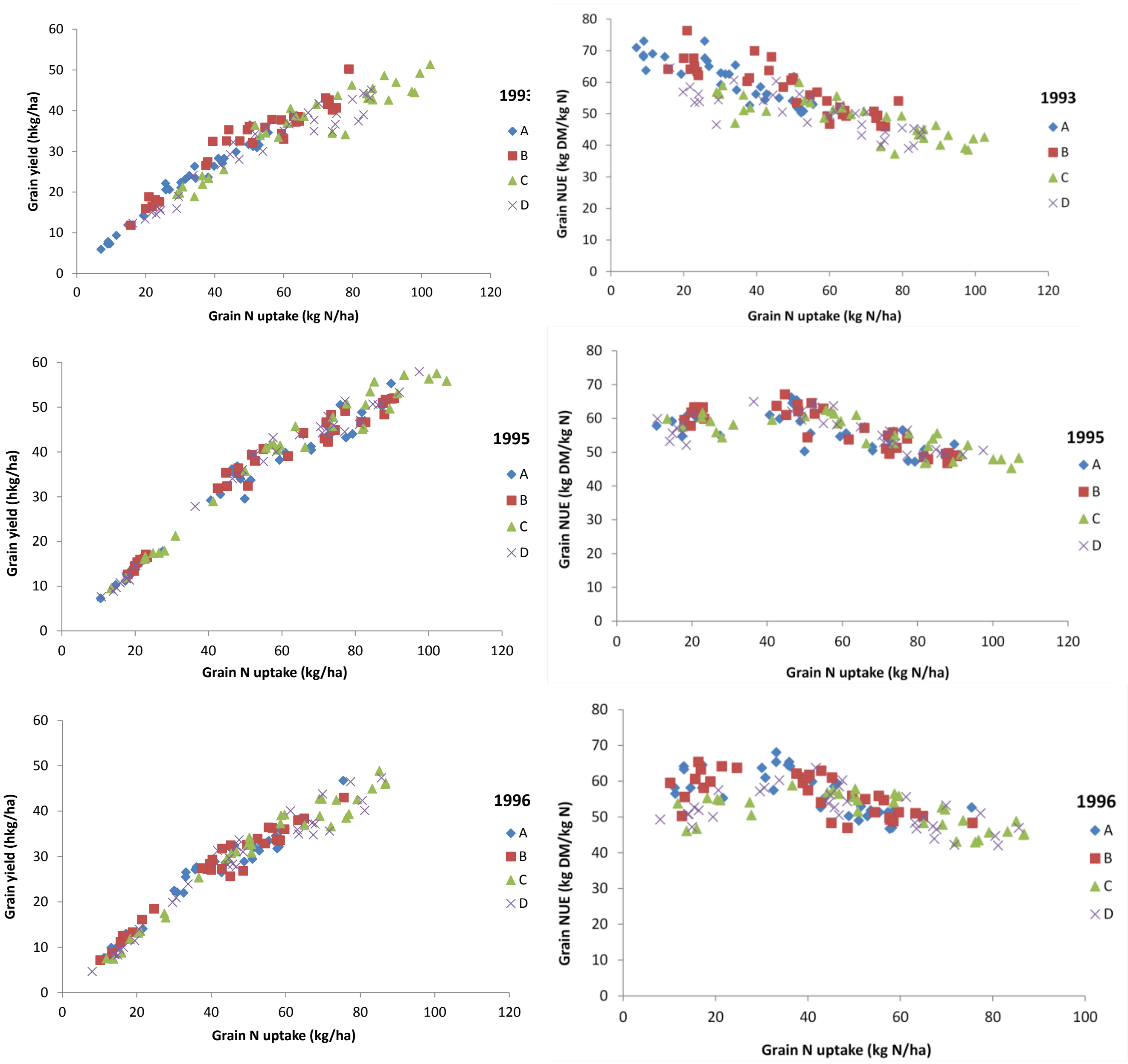

Figure 1. Dataset JYN. Grain DM yield (left) and grain nitrogen use efficiency (right) shown against grain N uptake for the years 1993, 1995 and 1996. The cropping year 1994 was very similar to 1995.

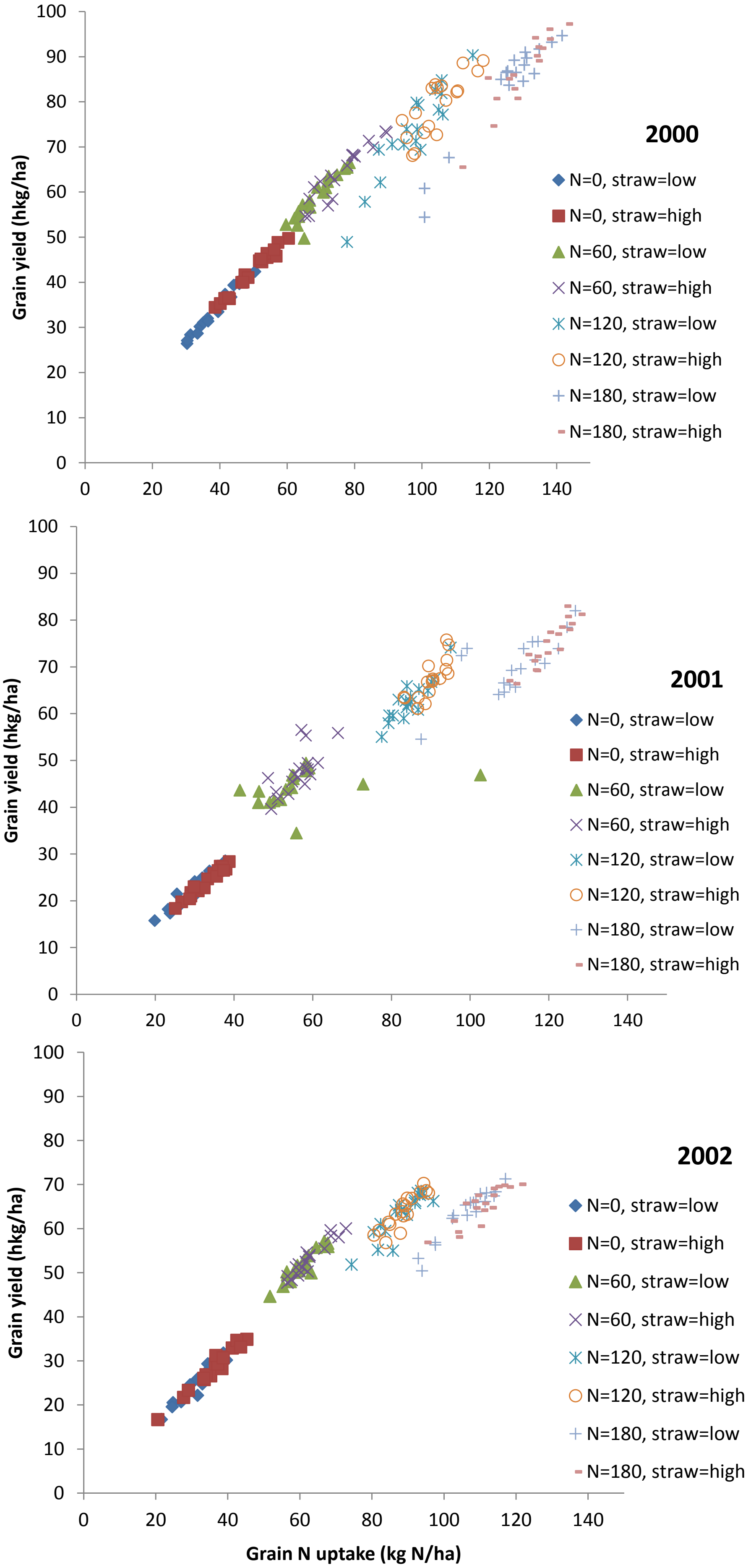

Fig. 2. Grain yield (DM w 15% water) versus grain N uptake (kg N/ha) at site ASK. Symbols show treatments (N input during test phase and straw input level during build-up phase). Low straw = 0 or 4 t/ha, High straw = 8 or 12 t/ha.

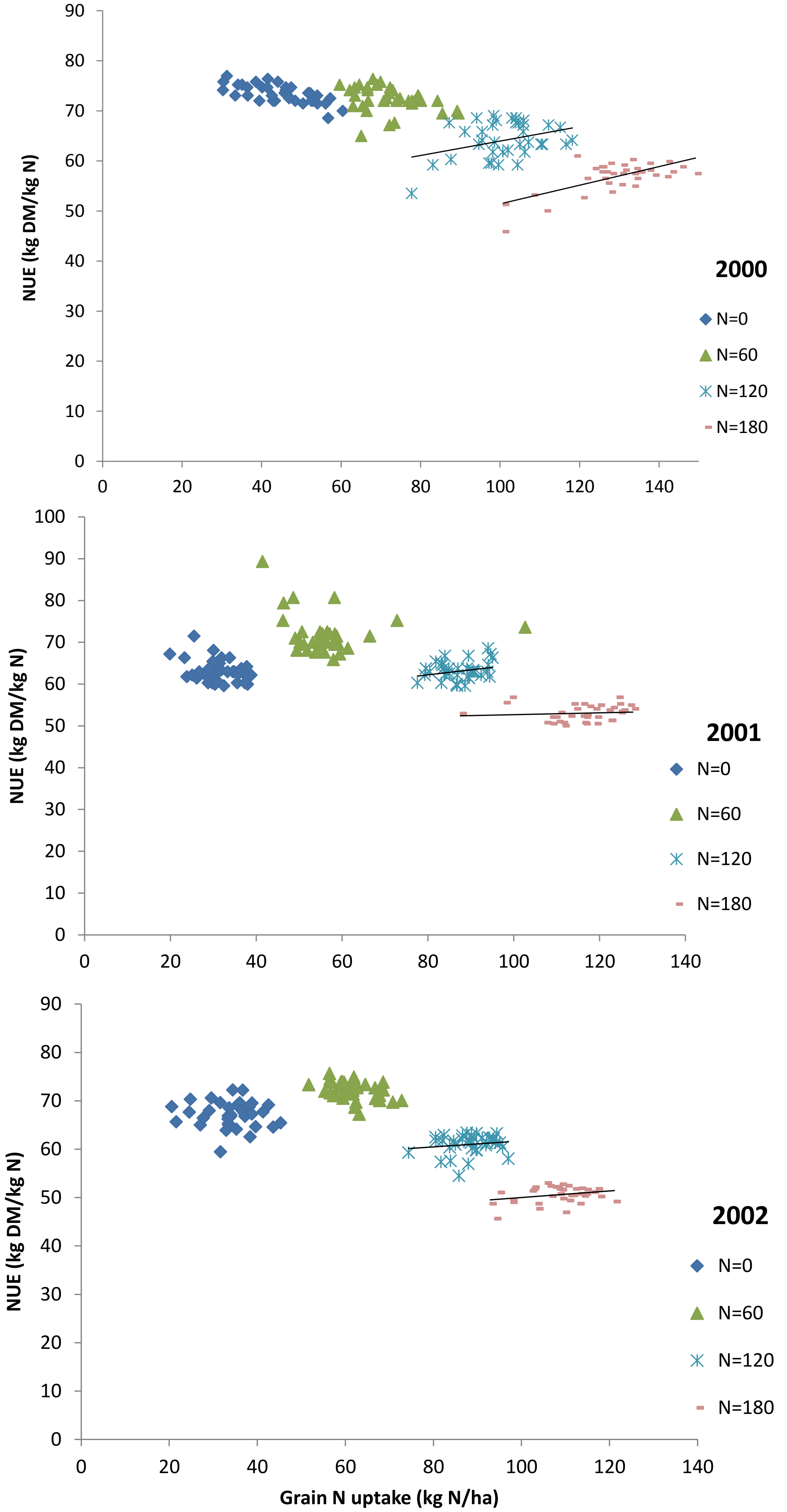

Fig. 3. Physiological NUE vs. grain N uptake at the ASK site. Black lines show trends that are opposite to the generally decreasing NUE with N uptake.

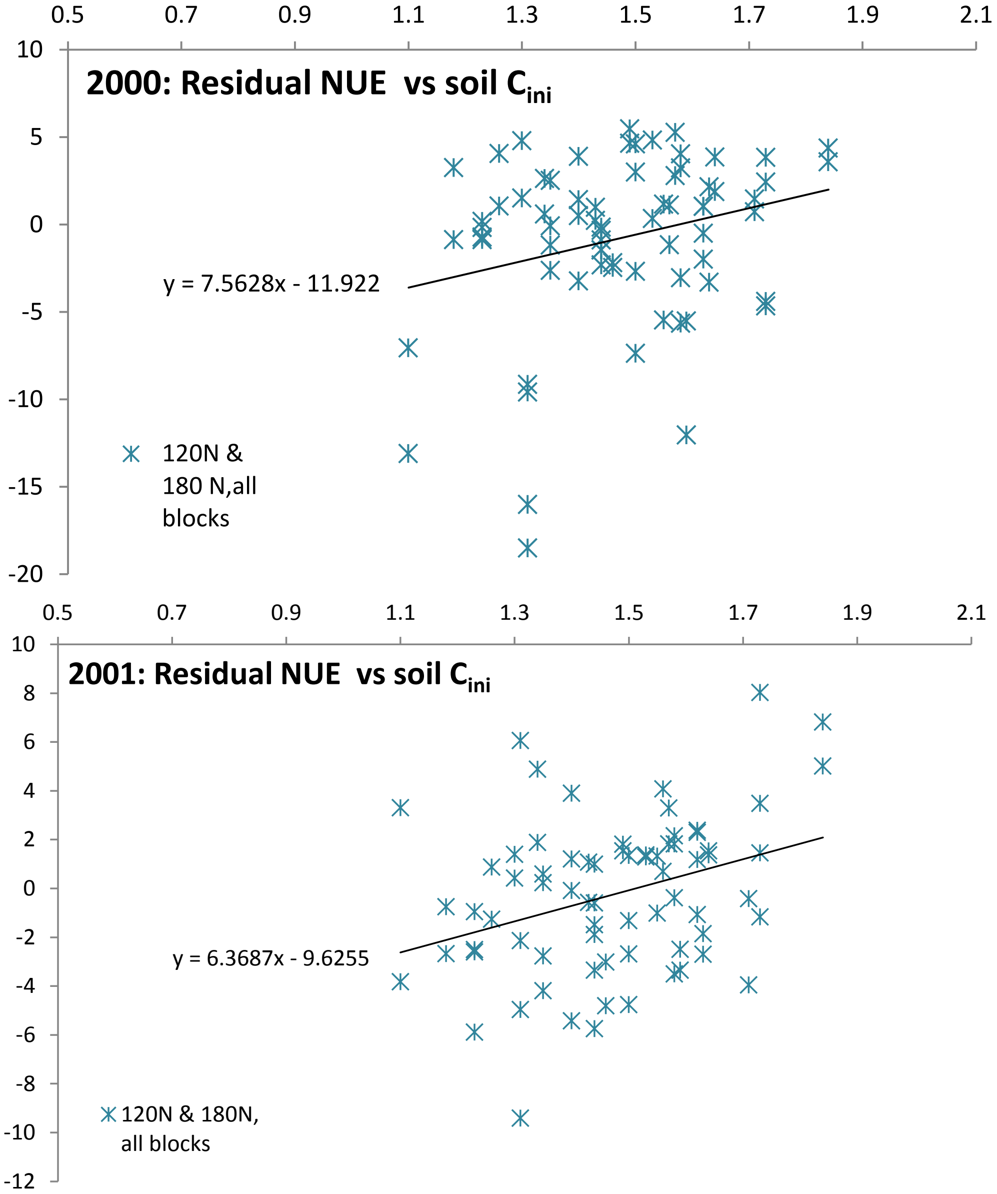

Fig. 4. Residual NUE (difference between observed and modelled value) shown against soil C content (%C) as observed before the test phase at the ASK site. Results from 2000 and 2001 are shown.

Table 3. Slopes [kg DM/(kgN %C)] of the relation between residual NUE (difference between actual and modelled physiological NUE) and the soil C content. The relations were tested for significance for input levels of 180N and for 120N and 180N bulked together.

N level	**Y 2000**	**Y 2001**	**Y 2002**
120 & 180N	7.6**	6.4**	4.4*
180 N	9.9**	7.8**	4.9

** Significant at 5% level
* Significant at 10% level

NUE residuals were plotted against soil C content observed before the test phase. Data indicated (Fig. 4) that grain NUE (at the high N fertilizer classes, 120 N and 180 N) was positively correlated to soil C content. Data was scattered and significance tests were made on the NUE-residual – SOC relationships. Results (Table 3) showed that the positive relationship was significant during all years if data from the 120 N and 180 N plots were merged in the analysis. If only data from the 180 N plots were analysed, the relation was significant during 2000 and 2001.

Yields at site ASK decreased over the three test years (figure 4). There was a similar decreasing trend in the national average yields of winter wheat for 2000-2001 (Statbank Denmark), however yields (at 180 kg N input) at site II in 2000 were higher than national yields while yields in 2002 were lower than national wheat yields. Two reasons for the yield decline at site ASK could be that i) the residual effects on yield of incorporated straw and catch crops during the accumulation phase were more exhausted during the third test year compared to the first test year (Thomsen and Christensen 2004) and ii) a cropping sequence effect (three years of continuous winter wheat following a spring barley) might have caused increasing crop failure in spite of pesticide treatments.
The general national decreasing yields over the three test years could be related to weather conditions. The first test year was the warmest and driest year at the unirrigated ASK site. It was also the year when the SOC effects were most prominent (visually, figure 2 and statistically, table 3). This could indicate that the SOC effects on yield could be related to soil water holding capacity, leading to diverting nitrogen utilization efficiencies in the field due to different soil moisture conditions in the drier test year.

To sum up, we analysed two datasets. According to dataset JYN, cereal yields and crop physiological nitrogen utilization efficiency (NUE) seemed to result from N supplies (N uptake) alone. However, according to dataset ASK, there was an additional trend in the cereal yield and NUE that was significantly correlated with SOC. For individual high N fertilizer rates at the ASK site, high N uptake rates were associated with relatively higher NUE at locations where SOC was also relatively high. The results do not prove that SOC caused the advantageous NUE, but as already explained we would expect nutritional effects (N release during the growing season) from SOC to have an opposite impact on NUE, namely a higher grain N% and thus lower NUE. If we assume an increase in NUE of 6 kg DM/kg N for an increase in SOC of 1% (based on table 3), and if we assume an N uptake of 120 kg N/ha (based on Fig. 2), an improved soil C status by 1% SOC would increase the yield by 7.2 hkg DM, or on the order of 10% (year 2001 yields at ASK). This indicates that the SOC effects identified here are significant and relevant from an agronomic point of view.

Johnston et al. (2009; section 4.3.1) reported from the Rothamsted experiments made in spring barley on Hoosfield. On Hoosfield, different SOM levels had developed due to farm yard manure (FYM) and fertilizer treatments. The plots were subdivided into subplots and four rates of fertilizer N were tested. After the introduction of cultivars with a high yield potential, yields on plots with a FYM history were always higher than yields without a FYM history. The authors stated that additional N, mineralised late in the growing season and deeper in the profile, contributed to the larger yield; but they also believed that much of the difference was due to SOM improving the soil structure.

The differences in NUE responses at site ASK compared to site JYN may be linked to the two soil types investigated. The soil type at site JYN was a coarse sandy soil. The soil type at site ASK was a sandy loam. Possibly the comparatively higher clay content in the ASK soil composes a soil type where increases in SOC have slightly more impact on cereal productivity. Considering Dexter ratio, or Fines/OC-ratio (Schjønning et al. 2012), both sites had adequate ratios below 20 (Fines/OC ratio at JYN around 4.7; Fines/OC ratio at ASK around 12), suggesting that the mineral fines of both soils were well 'saturated' with SOC.

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