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SHORT COMMUNICATION

Organic carbon stocks in topsoil and subsoil in long-term ley and cereal monoculture rotations

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Abstract

Soil organic C (SOC) in agricultural soils is affected by cropping system. In order to compare the impact of ley-dominated and cereal monoculture rotations on soil properties, a series of experiments was started in Sweden in the early 1980s. This study quantified the effect of rotations and N fertilisation on SOC stocks and microbial community composition. After 35 years, soil samples taken down to 50-cm depth at two sites with contrasting soil texture (clay and loam) were analysed for soil C and N, bulk density and phospholipid fatty acid (PLFA) content. Significant increases in SOC concentrations and stocks were found in the ley-dominated rotation compared with the cereal monoculture rotation, the difference being 0.36 and 0.59 Mg C ha^{-1} $year^{-1}$ in the topsoil (0–20 cm) for sites with the clay and loam texture, respectively, in average over N fertiliser levels. Nitrogen fertilisation increased SOC stocks significantly in the cereal monoculture, but not in the ley-dominated rotation. In the loam, SOC responses in the subsoil were almost as high as those in the topsoil, but they were insignificant in the clay soil. These results indicate that soil texture and structure can have a great impact on the potential of subsoils to sequester C, which requires attention when scaling up SOC sequestration rates for regional or global assessments.

Keywords Soil organic matter · Carbon sequestration · Subsoil · Crop rotation · Phospholipid fatty acids

Introduction

Soil organic matter (SOM) in soils has several functions linked to soil fertility, sustainability and quality (Hartemink et al. 2014). In fact, substantial amounts (27–77%) of soil organic C (SOC) can be present at depths greater than 20 cm (Harrison et al. 2011). Globally, SOC stocks down to 3-m depth are estimated to be 2300–3300 Pg C (Batjes 1996; Jobbágy and Jackson 2000; Tarnocai et al. 2009), which is three to four times the amount of C stored in the atmosphere (Lal 2013; Paustian et al. 2016). Land use and agricultural practices that lead to increased SOC stocks may play a major role in climate change mitigation through C sequestration, as for example advocated by the French minister Le Foll during the COP 2015 meeting in Paris by the so-called 4‰ initiative (http://4p1000.org). However, land use and management effects on subsoil C cycling are rarely measured (Jenkinson et al. 2008) and are not considered in regional and global C budgets (Crowther et al. 2016).

Changes in subsoil C content have been pointed out as a priority research topic (Swift 2001; Lorenz and Lal 2005). Literature reviews and meta-analyses on SOC sequestration show that experimental data on management practices such as tillage, N fertilisation, use of cover crops, crop residue incorporation and manure applications are almost solely available for arable topsoils (e.g. Alvarez 2005; Lehtinen et al. 2014; Maillard and Angers 2014; McDaniel et al. 2014; Meurer et al. 2018). However, the few existing studies addressing SOM changes in subsoil in a number of long-term field trials have shown that vegetation and crop management can have a significant impact on subsoil SOC, sometimes down to 1-m depth (Jenkinson et al. 2008; Syswerda et al. 2011).

Several mechanisms are involved in the SOC distribution within a soil profile, which can differ between ecosystems and species and within ecosystems (Jobbágy and Jackson 2000; Lorenz and Lal 2005). In particular, root-derived C, which

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contributes more to formation of stable SOM than aboveground crop residues and animal manure (Rasse et al. 2005; Kätterer et al. 2011), is a key input variable, although C input into subsoils can also occur through transport of dissolved organic C (DOC) from the topsoil and/or through bioturbation of litter/crop residues (Rumpel and Kögel-Knabner 2011). In agroecosystems, there are significant differences in root biomass allocation (Bolinder et al. 2007) and root distribution pattern between species down through a soil profile (Fan et al. 2016). For instance, total root biomass of perennial forage crops is typically at least threefold that of small-grain cereal crops (Bolinder et al. 2012). Consequently, higher SOC levels are consistently found under perennial crops (ley and grassland) or crops combined with catch crops than under annual crops (Chirinda et al. 2010; Taghizadeh-Toosi et al. 2014). Cropping systems producing more roots result in higher SOC in both the topsoil and upper subsoil (Kätterer et al. 2011; Kirchmann et al. 2013). However, a thorough understanding of the processes involved is still lacking.

Mean SOC stock estimates for mineral agricultural soils range from 54 Mg C ha^{-1} (0–15 cm) in Finland to 63 and 94 Mg C ha^{-1} (0–25 cm) in Denmark and Sweden, respectively (Andrén et al. 2008; Heikinen et al. 2013; Taghizadeh-Toosi et al. 2014). The SOC content in topsoil is higher in the Nordic countries than in other European countries, as illustrated by the recent LUCAS soil survey (Merante et al. 2017). Swedish agricultural land (about 3 Mha in 2014) is currently dominated by grass leys (30%) and grassland (15%), which may also explain the high SOC content (Poeplau et al. 2015a). The second largest proportion of agricultural area in Sweden is small-grain cereals, representing approximately 35%.

Positive effects on C sequestration of perennial leys compared with annual crops have been reported by many authors. For example, Carter and Gregorich (2010) found that SOC increased by 12.8 Mg C under 7 years of tall fescue (a perennial grass) compared with small-grain cereals in Eastern Canada. DuPont et al. (2014) measured paired perennial grassland and cropland wheat sites in Kansas, USA, down to a depth of 1 m and found that root biomass was three- to sevenfold greater in perennial grassland than in cropland. Based on literature data compiled by Soussana et al. (2010), it is estimated that between 71 and 129 g C m^{-2} $year^{-1}$ may be sequestered in grasslands on mineral soils in Europe.

The overall objective of the present study was to investigate the long-term (35 years) effect of two contrasting rotations (cereal monoculture and a rotation with 25% cereals and 75% perennial ley), with and without N fertilisation, on SOC distribution in the soil profile and microbial community composition. Two parallel series of Swedish field experiments started in the early 1980s, and in this study, sites with two different soil types (a loam and a clay) were sampled to a maximum depth of 50–60 cm. Specific objectives were to:

1. determine the extent to which N fertilisation rate and crop rotation affected topsoil and subsoil C concentrations and stocks,
2. identify the soil depth to which significant differences between treatments occurred, and
3. analyse the soil microbial community composition in topsoil and subsoil, in order to reveal the possible impact of management regime.

Materials and methods

Sites description and experimental design

Two long-term trials in Southern Sweden (Lönnstorp (55.67 °N, 13.10 °E) and Lanna (58.35 °N, 13.13 °E), initiated in 1981 and 1982, respectively) were investigated 35 years after the experiments started. Annual mean temperature is slightly higher at Lönnstorp (7.7 °C) compared to Lanna (6.1 °C) while the precipitation is almost the same (569 and 558 mm $year^{-1}$, respectively). A bigger difference is the soil, where Lönnstorp has 15% clay in the topsoil, and Lanna has 43% (Poeplau et al. 2015b). The Lönnstorp loam is located in a region with very fertile soils and the clay minerals are mainly illite, smectite and kaolinite (Kirchmann and Eriksson 1993).

On both sites, one 4-year crop rotation ('ley rotation') with 3 years of grass-clover ley sown as a mixture of meadow fescue, timothy and red clover (*Festuca pratensis*, *Phleum pratense* L. and *Trifolium pratense* L.), followed by 1 year of cereals, has been run in parallel with a continuous cereal (barley, oats or spring wheat) rotation ('cereal monoculture'). In the ley experiment, four N treatments (0, 50, 100 and 150 kg N ha^{-1} $year^{-1}$) are randomised within each of four blocks. Plot size is 105 m^2. Ley biomass is removed twice per year and straw is removed in the year when a cereal crop is grown. The same amount of N is applied to cereals in the ley rotation.

The cereal monoculture follows a split-plot design, with straw removal/retention as main plots randomised in each of four blocks and four N fertilisation treatments (0, 40, 80 and 120 kg N ha^{-1} $year^{-1}$) randomised within each main plot. In both systems, the total amount of phosphorus (P) and potassium (K) removed in harvested products in each treatment guides the amount of P and K applied every second year. An initial dose of 35 kg P and 65 kg K ha^{-1} was applied in the first and third year of the experiments. All fertilisers are applied as single doses at sowing in spring. In this study, we investigated the most contrasting N treatments, receiving no N and the highest rates, and in the cereal monoculture only plots with straw retention, since this is the most common management practice.

Soil sampling

Since the start of the field experiments in 1980 and 1981, samples from topsoil (0–20 cm) have been taken almost every fourth year, but only as general samples comprising four plots with the same treatment.

On 15–16 September 2015, soil samples were taken in layers to 50-cm depth at Lönnstorp and at 20 September 2016 at Lanna to 60-cm depth. For both sites, this coincided with the time of harvest of the ley after the third year in the rotation. Soil bulk density was also measured with triplicate cylinders (7.2-cm diameter) in topsoil (5- to 15-cm depth) in each plot. Bulk density in the cereal monoculture at Lönnstorp was not measured, since the site had already been ploughed. Occurrence of mineral particles larger than 2 mm was determined in cylinders from Lönnstorp. Content of gravel and stones at Lanna is reported to be only 2 mg g^{-1} at 0- to 20-cm and 1 mg g^{-1} at 30- to 60-cm depth (Kätterer et al. 2014a), and we therefore neglected this in the SOC stock calculations.

For chemical and biological analyses, soil samples were taken at five locations within each plot. Auger samples were sliced into eight or nine depth layers to 50- and 60-cm depth at Lönnstorp and Lanna, respectively. The samples were stored at 2 °C prior to analysis. Subsamples for phospholipid fatty acid (PLFA) analysis were stored in coolboxes before freeze-drying on the day after sampling. The samples for element analysis were dried and sieved at 2 mm.

Soil analyses

Soil C, N and pH were measured after drying the soil samples at 40 °C. Total C and total N were determined by dry combustion (LECO CNS Analyser; LECO Corporation, St Joseph MI 49085, USA). Based on previous experiences in this region (e.g. Kirchmann and Eriksson 1993), samples from Lönnstorp were also analysed for carbonates (subtraction method, after removing organic C at 550 °C). No carbonates are present at the Lanna site (e.g. Eriksson et al. 2016). For SOC analyses, dry combustion has been used since the beginning of the 1980s. Re-analysis of stored samples with a recent instrument showed good agreement with previous data, i.e. no bias from instrumentation. Soil pH (1:2.5 solid to liquid ratio) was determined with a glass electrode (MeterLab, PHM210 Standard pH Meter).

Calculations of SOC stocks

The SOC stocks in the 0- to 20-cm layer were calculated from SOC concentrations and bulk density (BD). To avoid overestimation of SOC stocks (Poeplau et al. 2017), the fraction of gravel and stones (mineral particles < 2 mm) was considered for Lönnstorp (not for Lanna):

$$SOC_{\text{stock}} = SOC\%_{\text{fine soil}} \times BD_{\text{fine soil}} \times \text{depth} \times \left(1 - f_{\text{gravel}}\right) \quad (1)$$

where f_{gravel} is the volume fraction of gravel and stones, which was calculated from the mass of gravel and stones in the sample divided by an assumed rock density of 2.6 g cm^{-3}. The bulk density of the fine soil fraction ($BD_{\text{fine soil}}$) was corrected for mass and volume of gravel and stones:

$$BD_{\text{fine soil}} = \frac{\text{mass}_{\text{sample}} - \text{mass}_{\text{gravel}}}{\text{volume}_{\text{sample}} - \text{volume}_{\text{gravel}}} \quad (2)$$

Changes in SOC stocks at Lönnstorp and Lanna over time were calculated from initial and final C concentrations and BD. Since BD at the start of the experiments was not measured, we used the close negative correlation between SOC and BD to estimate initial BD values. For SOC stocks below 20-cm depth, we approximated BD data assuming 10% above the average value over all treatments per site in the topsoil layer. This assumption is supported by data in a Swedish soil database (Kätterer et al. 2006). The content of gravel and stones measured in the topsoil was assumed to be the same throughout the profile. For the cereal monoculture at Lönnstorp where BD could not be measured in 2015, we assumed it to be 6% higher than in the ley rotation, corresponding to the relative difference in BD between the rotations at Lanna.

Since the mass of soil over a pre-defined depth differs between treatments with different BD, soil depth was adjusted when comparing treatment effects on SOC stocks. To account for differences in soil depth observed as different surface elevation in a non-mechanised field experiment (Kätterer et al. 2011), an equivalent soil mass approach (Ellert and Bettany 1995) was applied. Following this approach, the treatment with the lowest soil mass at each site was taken as reference mass in order to calculate the soil depth to which the same soil mass is distributed in the heavier soils of the other treatments. The equivalent mass concept was also used when comparing initial with final SOC stocks.

Carbon balances

We estimated the annual C inputs to soil for all sites, rotations and N levels from the measured forage (total aboveground harvested biomass) and cereal (grain) dry matter yields using plant C allocation coefficients (Bolinder et al. 2007). Concentration of C in all plant parts was assumed to be 0.45 g g^{-1}. The shoot-to-root ratio used to calculate belowground C inputs (root biomass) was 1.6, 3.1 and 7.4 for established forage, undersown forage and cereal monoculture,

respectively (Bolinder et al. 2007). These values were based on root studies to a median depth of 40 cm for cereals and 25 cm for leys. In order to calculate the belowground C inputs to 0- to 20- and 0- to 50-cm depth, we followed the method used by Kätterer et al. (2011) based on a Michaelis-Menten-type function for root distribution with a maximum rooting depth of 150 cm, and 50% of the root biomass being present in the upper 10-cm layer. This means that 71 and 89% of the roots are in the 0- to 20- and 0- to 50-cm soil layers, respectively, a root distribution that corresponds to that measured in Swedish field experiments (Hansson and Andrén 1987; Kätterer et al. 1993) and also agrees well with a recent review on root distribution patterns for agricultural crops in temperate regions (Fan et al. 2016). The proportion of extra-root (rhizodeposit) C was set to 65% (i.e. 0.65 × root biomass) for all crops (Bolinder et al. 2007).

The aboveground C input by forage crops (i.e. litterfall, harvest losses and senescence during winter) was considered to be 25 and 40% of total aboveground harvested biomass in the production years and last year of production, respectively (Bolinder et al. 2012). When straw yield was not measured, straw added to soil was calculated using a harvest index of 0.54, based on average data for spring cereals (barley, oats and spring wheat) estimated from 536 trials and experimental sites across Sweden (Adolfsson 2005).

PLFA analysis

Phospholipid fatty acids were analysed according to methods described by Börjesson et al. (2014). A total of 28 peaks were determined and quantified. Gram-negative bacteria were calculated as all PLFAs with one double-bond or a cyclic structure. All other branched PLFAs except 10-methylated were regarded as Gram-positive; 18:2ω6,9 was used to represent fungi, while PLFAs 10Me16:0 and 10Me18:0 were taken to represent Actinobacteria (Dungait et al. 2011). The mass of bacterial C was based on the sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, 17:1/a17:0, cy17:0, 17:0 18:1ω7c and cy19:0/19:1. This was converted to C in soil samples using a factor of 2.75 μg C $nmol^{-1}$ PLFAs and a factor of 84.7 μg C $nmol^{-1}$ PLFA 18:2 for fungi (Williams et al. 2013).

Results

Yield and C input from residues

At both sites, harvest data showed that yield of grass-clover in the ley rotation was generally high even when no N was applied (Suppl. Table S1). Nitrogen fertilisation was less important for yield of the grass/clover leys, as low or no N input was compensated for by an increasing proportion of N-fixing clover (Karlsson 2013). The yield increase due to N fertilisation (150 N) was only 16% at Lönnstorp and 41% on the Lanna clay. Thus, unfertilised leys probably provided similar amounts of annual C input as those fertilised with N. Cereal yields were higher at Lönnstorp than at Lanna, especially for the intervening harvests of cereals in the ley rotation indicating that the Lönnstorp loam is more fertile than the Lanna clay (Suppl. Table S1).

At both sites, total mean annual C input to soil from plant residues was much higher for the ley rotation than for that cereal monoculture cropping (Suppl. Fig. S1). In leys, input of C by roots dominated, whereas in cereal monoculture straw was the main residue since it was left in plots. Belowground C input (0–50 cm) amounted to 1.7–2.5 Mg C ha^{-1} in leys and 0.25–1 Mg C ha^{-1} in cereals.

Changes in soil organic C concentrations

In the topsoil (0–20 cm) at both sites, SOC concentrations increased in the ley rotation but decreased under cereal monoculture. Relative SOC differences between ley and cereals increased with time, amounting to 67% at Lönnstorp and 44% at Lanna after 35 years (Fig. 1). In the cereal monoculture, mineral N fertilisation retarded the SOC decline compared with no N. In leys, no significant difference on SOC was observed between N fertilisation and no fertilisation (see also Suppl. Table S2).

With increasing soil depth, concentrations of SOC generally decreased but the two sites showed different patterns. At Lönnstorp, both rotation and N fertilisation affected SOC below 20 cm, whereas at Lanna there was no impact of treatments on subsoil organic C (Fig. 2; Suppl. Table S2). Below the plough layer at Lanna (22.5 cm, Suppl. Table S2), no significant differences were detected. At Lönnstorp, differences between treatments measured in the topsoil continued down the profile, showing the same pattern as in the topsoil. It was also noted that the C:N ratio was 1–2 units lower in the Lönnstorp profile and decreased with depth, while the ratio was consistently above 10 in the Lanna soil (Suppl. Table S3).

Bulk density and soil pH

Bulk density was somewhat higher in the unfertilised than in the fertilised treatments, but the differences were not significant. On average, bulk density in the ley topsoil at Lönnstorp was 1.46 (s.d. 0.05) in unfertilised and 1.41 (s.d. 0.06) g cm^{-3} in fully fertilised treatments. In the cylinder samples from Lönnstorp, mineral particles larger than 2 mm amounted to 6.5 ± 2.8%. Thus, correcting for gravel and stone content for this site slightly lowered the fine-earth soil fraction content, resulting in BD values of 1.43 and 1.38 g cm^{-3} for unfertilised and fully fertilised, respectively, at Lönnstorp. Mean BD values at Lanna were

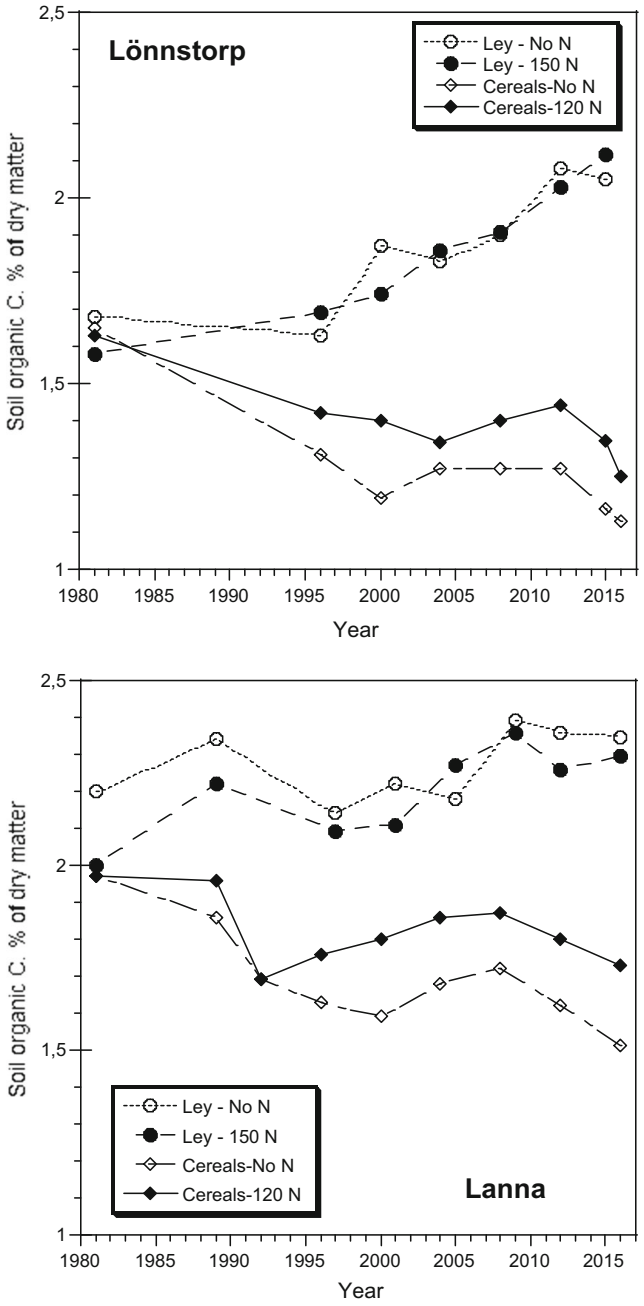

Fig. 1 Development of C in topsoil (0–20 cm) since start of the experiments, with different N fertilisation regimes (kg N ha^{-1})

lower than those at Lönnstorp, 1.34 and 1.33 g cm^{-3} in unfertilised and fully fertilised, respectively. Cereal monoculture at Lanna had significantly (*t* test; $p = 0.016$) higher BD values (1.42 and 1.41 g cm^{-3} for no N and 150 kg N, respectively) than the ley rotation.

At Lönnstorp, soil pH values have decreased over the 35 years of the experiment in both cereal and ley cropping, by on average 0.53 units. At Lanna, pH values have remained stable in the cereal monoculture, but have declined in the ley rotation, at a similar rate in both treatments, from 6.5 to 5.6 (Suppl. Fig. 3).

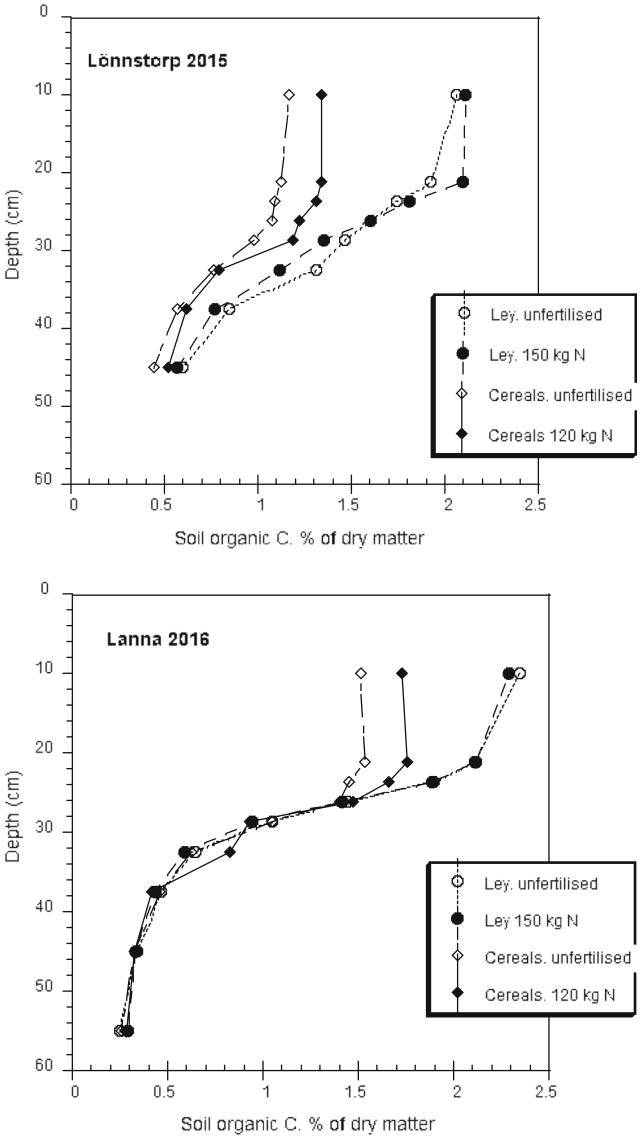

Fig. 2 Carbon in soil profiles. Each point represents $n = 4$ plots of the same treatment (cf. Suppl. Table 2 for statistics)

Soil organic C stocks

Stocks of SOC after 35 years were significantly higher in the ley rotation than in cereal monoculture at both sites (Table 1). Nitrogen fertilisation resulted in significantly higher SOC stocks in the cereal monoculture, while there was no obvious effect of N fertilisation on SOC stocks in the ley rotations. According to our calculations, the change in SOC stocks over sites and treatments ranged from −0.36 to +0.35 Mg ha^{-1} $year^{-1}$ in the topsoil layer (0–20 cm) and from −0.68 to +0.47 Mg ha^{-1} $year^{-1}$ in the 0- to 50-cm layer. The most striking finding was that SOC stocks in the subsoil at Lönnstorp were strongly affected, whereas no such changes were observed at Lanna. Furthermore, although yield differences between no N and N-fertilised treatments were higher at Lanna than at

Table 1 Initial (1980 or 1981) and final (after 35 years = 2015 or 2016) SOC stocks (Mg C ha^{-1}) and mean annual changes (ΔSOC) in the cereal monoculture and ley rotations with and without N fertilisation at the two sites. SOC stocks at 0–20 cm with different letters were significantly different. Annual ΔSOC could not be tested statistically since only mean values per treatment were available for initial SOC concentrations. Testing final SOC differences to an equivalent depth of 50 cm was not meaningful since bulk density values were not available for the subsoil but were estimated as described in the text

	Lönnstorp (1980–2015)				Lanna (1981–2016)			
	Cereal mono		Ley rotation		Cereal mono		Ley rotation	
	−N	+N	−N	+N	−N	+N	−N	+N
0–20 cm								
Initial	46.3	45.8	47.2	44.4	54.0	54.0	60.2	54.8
Final*	33.8 c	39.1 b	56.9 a	56.5 a	42.8 b	48.7 b	62.7 a	60.7 a
ΔSOC	− 0.36	− 0.19	0.28	0.35	− 0.32	− 0.15	0.07	0.17
20–50 cm (eq.)								
Initial	43.0	42.9	42.9	42.9	36.1	36.1	36.2	36.2
Final	31.7	36.2	49.2	47.3	33.6	36.3	37.4	36.4
ΔSOC	− 0.32	− 0.19	0.18	0.13	− 0.07	0.01	0.03	0.01
0–50 cm (eq.)								
Initial	89.3	88.7	90.1	87.3	90.1	90.1	96.4	91.0
Final	65.5	75.3	106.1	103.8	76.4	85.0	100.1	97.1
ΔSOC	− 0.68	− 0.38	0.46	0.47	− 0.39	− 0.15	0.11	0.17

*Values within sites followed by different letters are significantly different (Tukey-Kramer HSD, $\alpha = 0.05$)

Lönnstorp, differences in SOC stocks were lower at Lanna than at Lönnstorp.

At both sites, total mean C input from crop residues correlated well with changes in SOC stocks in both the 0- to 20- and 0- to 50-cm layers ($R^2 = 0.67$ and 0.61, respectively; $N = 8$, data not shown). However, differentiation between aboveground and belowground inputs revealed that aboveground C input was not significantly correlated with SOC stock changes ($p > 0.05$). On the other hand, high correlations between estimated belowground C inputs and SOC stock changes were found in both the topsoil and the 0- to 50-cm layer (Suppl. Fig. 2), suggesting that SOC changes were driven by belowground C inputs. According to the regressions, an annual input rate of 0.9 and 1.3 Mg C is required for maintaining present C stocks at Lanna and Lönnstorp, respectively (Suppl. Fig. 2). According to analysis of covariance, the interaction between site and belowground inputs significantly contributed to explain the variance in observed SOC changes. Thus, SOC responses to a certain amount of belowground input differed significantly between sites.

The long-term effect of continuous ley compared with cereal monoculture, averaged across N treatments, was an increase in topsoil (0–20 cm) SOC stocks of 0.36 and 0.59 Mg C ha^{-1} $year^{-1}$ at Lanna and Lönnstorp, respectively. This is close to the estimated mean difference of 0.52 Mg C ha^{-1} $year^{-1}$ between ley-arable systems and annual cropping systems reported for Nordic conditions (Kätterer et al. 2013). When the subsoil was also included, ley rotations accumulated between 0.41 and 1.0 Mg ha^{-1} $year^{-1}$ more SOC at Lanna and Lönnstorp, respectively, compared with cereal monoculture (Table 1). Consequently, the subsoil accounted for 12–41% of the increase in SOC stocks in the 0- to 50-cm layer, similar to what has been reported for another long-term experiment at Lanna (Kätterer et al. 2014a).

PLFAs

The PLFA analysis showed a higher amount of microbial biomass at Lanna than at Lönnstorp, reflecting the organic C content of the soils (Suppl. Table S4). Strong positive correlations between total PLFAs and SOC were found for soils at both sites ($r = 0.975$ at Lönnstorp, $r = 0.896$ at Lanna). Analysis of microbial community composition (% of total moles PLFA) showed a significant decrease in fungi with depth at Lönnstorp, while Actinobacteria increased. This effect seemed also to be present at Lanna, but was not significant at that site (Suppl. Table S4).

Examination of the ratios of the PLFAs cy17 and cy19, known as stress indicators, and their precursors 16:1ω7 and 18:1ω7 showed that these were much higher in the subsoil samples and that, in particular, the cy17/16:1ω7 ratio in Lanna subsoils was higher than other ratios, with values around 1.40 (Suppl. Table S5).

Discussion

Carbon balance

The amount of aboveground plant residues was relatively high in the cereal monoculture rotation, since the straw was left in the experimental plots. However, the contribution of straw to total plant C inputs was lower than the belowground input by leys. It has been previously found in other Swedish long-term experiments that the fraction of C input from belowground residues entering the SOC pool (i.e. humification coefficient) is at least twice that from aboveground crop residues (Kätterer et al. 2011, 2014a; Menichetti et al. 2015; Poeplau et al. 2015b; Ghafoor et al. 2017). The higher C retention from belowground residues can explain the higher SOC contents observed for leys.

Yields were more than twice as high for the fertilised cereals (120 N) compared with the unfertilised treatments, and annual loss of SOC in the topsoil (0–20 cm) was 0.17 Mg C ha^{-1} lower in the fertilised than that in the unfertilised cereal monoculture at both sites (Table 1). Thus, the amount of C sequestered in the topsoil through N fertiliser (170 C/120 N) was 1.4 kg C per kg N applied, which is similar to values reported for topsoil in other Swedish long-term field

experiments (Kätterer et al. 2012, 2014b). However, when subsoil layers were also considered, C sequestration was almost twice as high at Lönnstorp. To an equivalent depth of 50 cm, the positive effect of N fertilisation on SOC was 0.24 and 0.30 Mg C ha^{-1} $year^{-1}$ at Lanna and Lönnstorp, respectively, corresponding to 2.0 and 2.5 kg C kg^{-1} N, respectively. Thus, subsoils should be considered when evaluating treatment effects on SOC storage (Kirchmann et al. 2013).

It is possible that the amount of belowground C input from unfertilised cereals was underestimated with the approach developed by Bolinder et al. (2007), since it has been well documented that unfertilised spring cereals can allocate about 35% more C belowground than fertilised spring cereals (e.g. Welbank and Williams, 1968; Welbank et al. 1974; Hansson et al. 1987). However, even on correcting for this (e.g. using a shoot-to-root ratio of 5.6 instead of 7.4 for cereals), belowground C inputs in absolute terms were still much lower for the unfertilised cereals than for the N-fertilised cereals (data not shown).

Nitrogen fertilisation was less important for yield of grass/clover leys, as low or no N input was compensated for by an increasing proportion of N-fixing clover. Thus, unfertilised leys provided similar amounts of annual C input as those fertilised with N.

C inputs from roots and subsoil C differences

The high SOC content of the subsoil (≥ 20 cm) in the Lönnstorp loam (Figs. 1 and 2) in both the ley and cereal monoculture rotations indicates a relatively high proportion of roots entering the subsoil. However, no significant differences in subsoil C were found for the Lanna clay. Results similar to those in Lanna were also found at another Swedish site where four crop rotations did not affect SOC in the subsoil despite great treatment differences in the topsoil (Jarvis et al. 2017). Similarly to our data for the Lanna site, Syswerda et al. (2011) found no significant difference between annual and perennial crops in C sequestration in subsoil samples (41–100 cm), despite taking numerous samples, when comparing cropping systems in a 12-year-old field experiment in Southwest Michigan, USA.

A possible reason for less subsoil rooting at Lanna could be the very low P content measured in the upper subsoil layers (Andersson et al. 2013). Another reason could be the difference in soil texture and structure. Lower root-to-shoot ratios have been reported for fine-textured compared with more coarse-textured soils (Poeplau and Kätterer 2017), which corroborates our results for the clay at Lanna and the loam at Lönnstorp. Pierce et al. (1983) estimated limiting and critical bulk density values for root development in soils with different textures. For the clayey soil at Lanna, a bulk density of 1.49 g cm^{-3} would stop root growth. The value measured in the topsoil was 1.46 g cm^{-3} and higher values exceeding the critical limit for root growth were found in the subsoil. Temporary anoxic condition in the dense subsoil may also have contributed to restrictions for root growth as indicated by mottles in the subsoil profile at Lanna. Thus, restrictions on root growth in the subsoil at Lanna may explain the lower C content in lower parts in that profile compared with Lönnstorp (Fig. 2). The higher C:N ratio at Lanna also indicated a slower humification process in the Lanna profile (Suppl. Table S3). Clay could protect SOC against microbial degradation (Ladd et al. 1996), but in our study the C input by roots seemed to be more important.

PLFAs

The difference in microbial community composition was more prominent between topsoil and subsoil within sites than between sites, crop systems or fertiliser levels. A major reason was the higher abundance of fungi in topsoil. In subsoil samples, there were no differences that could indicate differences between the sites concerning microbial turnover of organic matter, but the elevated cy17/16:1ω7 ratio of around 1.40 in Lanna subsoil may indicate a stress situation, which could be due to low oxygen levels or nutrient deficiency (Kieft et al. 1997; Trögl et al. 2015), but not to pH since these values are high in the subsoil (around 7.0; Andersson et al. 2013).

Conclusions

Compared to the cereal monoculture, the ley rotation had significantly higher SOC concentrations and stocks in the topsoil at both sites. Nitrogen fertilisation increased SOC stocks significantly compared with no N fertilisation in the cereal monoculture but not in the ley-dominated rotation, where N fixation may have compensated for lack of fertiliser N inputs. On average, N fertilisation of cereals resulted in C sequestration in topsoil of 1.4 kg C per kg N applied. When including subsoil sequestration, this figure increased to 2.2 and 2.5 kg C per kg N applied at the two sites studied. Soil texture affected C sequestration. In loam soil, SOC changes in response to rotation were threefold higher than in clay soil. Moreover, rotation affected SOC stocks down to 50-cm depth in the loam soil, whereas in the clay soil only the plough layer was affected. Differences in soil microbial community composition between topsoil and subsoil were greater than differences between sites. The main reason was that the fungal/bacterial ratio was consistently higher in topsoil than subsoil. Rotation and N fertilisation had no obvious impact on the composition of the microbial community. The results show that management-induced changes in subsoil organic C stocks in Swedish agricultural soils are not negligible and should be considered in C accounting schemes. However, the magnitude of

these C stocks changes seems to be site-specific and depending on physical soil properties rather than on microbiological processes. Since it is not well known how widespread compacted soils are, future research should focus both on investigating the extent of the problem and on management techniques for improving the potential of soils for crop production.

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