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# Long-term ley and manure managements have consistent effects on microbial functional profiles and organic C groups across soils from a latitudinal gradient

Andong Shi<sup>1</sup> · Naoise Nunan<sup>1,2</sup> · João Figueira<sup>3</sup> · Anke M. Herrmann<sup>1</sup> · Johanna Wetterlind<sup>4</sup>

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## Abstract

Soil organic matter (SOM) is important in maintaining soil fertility and other ecosystem functions. Yet, land management in intensive agriculture has caused SOM level to decrease, with knock-on effects for soil fertility and quality. Therefore, land management options that ensure that SOM is not depleted and that soil functions are better sustained are of increasing interest. However, there is limited knowledge on how different land managements affect the composition of SOM and associated microbial functional profiles. Twelve long-term field experiments, covering a wide range of climatic zones and soil types, were selected in Sweden. They focused on the role of combining ley in crop rotations with the manure application (livestock farm), as opposed to the management without ley and receiving only inorganic fertilizer (arable farm). In ten out of the 12 study sites, livestock farm management tended to have higher proportions of aliphatic and double bonded C groups, as estimated by mid-infrared spectroscopy. This was further confirmed by <sup>13</sup>C NMR analysis, which found greater proportions of O-alkyl and di-O-alkyl groups and less aromatic C in livestock farm than arable farm management in five of the eight sites analyzed. The changes in SOM composition were reflected in microbial functional profiles across many sites: soils from livestock farm management utilized more carbohydrates and amino acids, while polymer and aromatic compounds were associated with arable farm management. Overall, shifts in both microbial functional profiles and SOM composition showed great consistency across geographical and climatic zones. Livestock farm management maintained higher levels of microbial functional diversity and were associated with higher proportions of “reactive” C functional groups. Our investigation demonstrates that livestock farm management could maintain soil fertility over the long-term via the changes in SOM composition and the regulation of microbial functional profiles.

**Keywords** DRIFT, Microbial functional diversity · Mid-infrared spectroscopy · Latitudinal gradient · Ley in crop rotation · Livestock farm management · Long-term experiment · <sup>13</sup>C solid-state CP/MAS NMR

## 1 Introduction

Soils are essential and limited resources and provide ecosystem services to human societies; thus, it is vital to manage soil sustainably. In modern agriculture, it is especially critical to maintain soil at a healthy and functioning level (Diacono and Montemurro 2010; Rasmussen et al. 1998). Often, the focus has been on the short-term benefit of maximizing crop productivity, and many important functions that soils provide have been neglected (Gaba et al. 2015), e.g., soils as a carbon (C) sink; decomposition of pollutants; and mitigation of the impact of climate change (Hoffland et al. 2020). A better understanding of how soil ecosystem services are influenced by agricultural practices demands a thorough examination of both soil organic matter (SOM)

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characteristics and soil microbial community functioning under different land managements. This is all attributed to the critical role of SOM in soil chemical, physical, and biological processes (Audette et al. 2021; Lehmann and Kleber 2015), as well as the interactions among all these processes. In fact, SOM is often regarded as the most reliable indicator of soil fertility and quality (Hoffland et al. 2020; Lal 2020). Therefore, changes in SOM properties, i.e., its chemical composition, may also alter the activity of microbial communities. Soil microbial communities also affect the decomposition rates of organic materials entering the soil, thus altering the composition of SOM (Audette et al. 2021). Given the critical importance of SOM in soil fertility, both the chemical composition and soil microbial activity could have an impact on soil quality and health over the long-term. Consequently, a finer understanding of the impact of management practices on soil fertility and quality requires that both the composition of SOM and microbial functioning be monitored.

The chemical composition of SOM is very complex, because SOM is a mixture of materials of plant and microbial origin that are consistently subjected to microbial processing. This complexity has been revealed using well-established spectroscopy techniques, e.g., infrared (Wetterlind et al. 2008, 2022; Zimmermann et al. 2007; Soriano-Disla et al. 2014) and nuclear magnetic resonance (NMR) (Leifeld and Kögel-Knabner 2005; Kögel-Knabner 1997; Kögel-Knabner and Rumpel, 2018; Baldock et al. 1997). The changes in SOM content have been associated with shifts in specific C functional groups among various long-term fertilization practices (Kaiser et al. 2007). Similarly, it has been reported that the addition of exogenous organic matter increased “reactive” C functional groups, e.g., O-alkyl groups, as opposed to aromatic groups (Mao et al. 2008). Hence, land management-induced changes in SOM content and quality can potentially be monitored by shifts in different C functional groups. Furthermore, the composition of SOM also affects the activity and shapes the structure of soil microbial communities (Brady and Weil 2008), which, in turn, are responsible for the mineralization and transformation of SOM (Hoffland et al. 2020). Changes in SOM composition, owing to different land management practices, could result in changes in soil microbial activity. Over time, such interactions may change the equilibrium in soil C dynamics, thus leading to differences in SOM content. However, such changes in SOM content can only be detected over the long-term, due to the slow rate at which SOM changes or accumulates (Smith 2004; Guillaume et al. 2021). Therefore, long-term field experiments (LTFE) with different land management practices provide a valuable platform for investigating the changes in SOM content.

Soil microbial communities can adjust their metabolism and physiology to changes in soil conditions (Schimel and

Schaeffer 2012) and, depending on the conditions, such as those under different land management practices, express different sets of functions (Schimel and Hättenschwiler 2007). Microbial functional diversity, as measured by catabolic profiling (Chapman and Koch 2007), integrates both microbial genotypic diversity and functioning (Escalas et al. 2019; Zak et al. 1994) and represents the metabolic capacity of the overall microbial community to a wide range of substrates, varying in molecular size and complexity. However, little is known about how different long-term management practices influence microbial functional diversity profiles and how the influence is related to the shift in chemical composition of SOM.

The effect of including ley in crop rotations has been shown to improve soil physical and biochemical properties (Bolinder et al. 2010; Jarvis et al. 2017; Prade et al. 2017). It has also been suggested that reduced soil disturbance, longer growth periods, and greater rooting depth and density associated with ley can stimulate soil microbial activities (Kätterer et al. 2012; Albizua et al. 2015; Prade et al. 2017; Martin et al. 2020). Indeed, it was found that soil organic C content was proportional to the number of years with ley cropping in 6-year crop rotation systems in Northern Sweden (Bolinder et al. 2012). Besides the influence of ley in rotations, livestock farming also results in the application of farmyard manure to the soils. Extensive studies have shown a fertilization effect (e.g., chemical fertilizers vs additional organic manure application) on plant performance and soil properties over the long-term (Gerzabek et al. 1997; Zhong et al. 2010; Liang et al. 2012; Lin et al. 2019; Gross and Glaser, 2021). Similarly, greater microbial respiration rates derived from the addition of multiple substrates were also found in treatment with higher organic matter input (Bongiorno et al. 2020). Overall, manifest benefits in both soil structure and the functioning in microbial communities have been found after animal manure applications (Diacono and Montemurro 2010; Dignac et al. 2017). Hence, the addition of manure not only supplies substrate to soil microbial communities, but also alters the soil environment where microbes inhabit, both of which would lead to changes in microbial functional diversity. Due to the combination of ley in crop rotations with the application of manure, we would expect an increase in microbial functional diversity in the fields of livestock farms. In the study by Martyniuk et al. (2019), both soil organic C and microbial biomass C concentration were largely increased (>30 %) by manure addition in crop rotation system with grass-clover ley, compared to the system of only maize-wheat-barley-maize and without the application of manure. Despite extensive studies on the individual effects of either the inclusion of ley in crop rotations or the addition of animal manure on SOM dynamics, a systematic investigation on the combined influence of both on SOM composition and microbial functional diversity

has, to our knowledge, not been carried out. Furthermore, it has been shown that many abiotic factors, e.g., mean annual temperature, precipitation, strongly influence SOM formation and accumulation (Kirschbaum 1995; Dai and Huang 2006; Conant et al. 2011). For example, it has been reported that SOC content is positively correlated with latitude and negatively with mean annual temperature (Dai and Huang 2006). Temperature-controlled processes are the primary explanations for the substantial amount of soil C pools in high altitude regions, e.g., reduced substrate availability or limited microbial activity (Conant et al. 2011). It is believed that the increases in SOC concentration are minimized under conditions where microbial decomposition is favored, such as high precipitation and temperature (Govaerts et al. 2009). Yet, no systematic investigation has been conducted on how the changes in both soil microbial functional diversity and SOM composition owing to different land management are further affected by various climatic and geographic factors.

Twelve LTFEs, established between 1957 and 1966 in Sweden, were selected. These trials focus on the effects of combining ley in crop rotations with manure applications (abbreviated as “livestock farm” management hereafter) versus rotations without ley and using only inorganic fertilizers (“arable farm” management). Here, we present an investigation of the influence of distinct long-term land management practices on soil organic C, total nitrogen (N) content, on the chemical composition of SOM, and soil microbial functional diversity. The objectives of the study were to: (1) evaluate the effect of long-term livestock farm management on soil properties across multiple LTFE trials and (2) examine if there was a link between the changes in microbial functional profiles and the shift in SOM composition. Moreover, the 12 trials cover a wide range of climatic zones (spanning at least 10 degrees in latitude) and soil types, allowing an examination of the interactions between geographic, climatic, or edaphic factors and management practices. We tested the following hypotheses: (1) soil organic C content and microbial functional diversity are higher in livestock farm management, compared to the arable farm management and (2) changes in (i) microbial functional diversity and (ii) SOM chemical composition owing to different long-term land managements will be further influenced by geographical and climatic factors.

## 2 Materials and methods

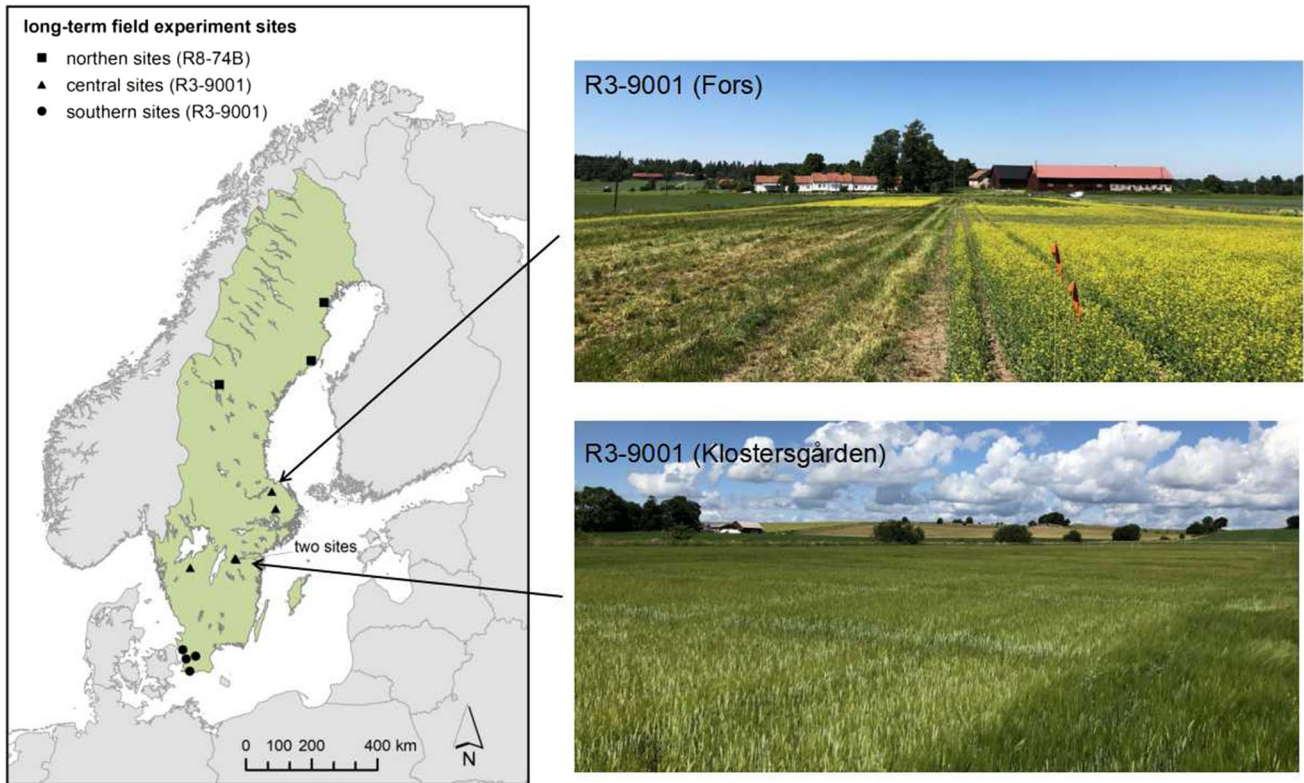
### 2.1 Long-term field experiment (LTFE) sites and experimental design

Samples were taken from two of the long-term field trial series of the Swedish University of Agricultural Sciences (SLU) (“R3-9001”: Plant nutrition and soil fertility; and

‘R8-74B’: Crop production systems in Northern Sweden). They include 12 experimental sites covering a wide range of soil types and spanning at least 10 degrees in latitude (Fig. 1). For more detailed geographical, climatic information and soil texture and classification, see Table 1 (more detailed information about the two LTFEs is included in supplementary file). In this study, the 12 sites were divided into three groups: (i) “southern sites” (including Fjärdingslöv, Ekebo, Orupsgården, and Borgeby from the R3-9001 series), (ii) “central sites” (Klostergården, Högåsa, Kungsången, Fors, and Bjertorp from the R3-9001 series), and (iii) “northern sites” (Ås, Röbbäcksdalen, and Öjebyn from the R8-74B series).

Two land management systems were compared: one including ley and farmyard manure (“livestock farm” management) and the other without ley and only using mineral fertilizers (“arable farm” management). The crop rotations and the application rates of manure and inorganic fertilizer are provided in Table 2. The experimental design differed slightly among site locations, with a 4-year crop rotation at the southern sites (barley-ley-winter wheat-sugar beet or barley-linseed-winter wheat-sugar beet in the livestock farm management and arable farm management, respectively). The manure application rate was 25 ton ha<sup>-1</sup> once every 4 years in livestock farm management. The central sites had 2 years of ley in a 6-year rotation of barley-ley-ley-winter wheat-oat-winter wheat in livestock farm management, compared with barley-oat-linseed-winter wheat-oat-winter wheat in arable farm management. The application rate of manure slurry was 30 ton ha<sup>-1</sup> every 6th year in the former. In the northern sites, the livestock farm management had a 6-year rotation of barley-ley-ley-pea (or oat)-potato-ryegrass with a manure slurry application rate of 20 ton ha<sup>-1</sup> year<sup>-1</sup>. The arable farm management had a 3-year rotation of barley-barley-pea (or oat) or barley-barley-potato.

Both long-term field experiment series investigated the influence of either using only mineral fertilizer (N, phosphorus, potassium) (“arable farm” management) or mineral fertilizer combined with applications of organic manure (“livestock farm” management) on crop yield since the start of the LTFEs (between 1957 and 1966). The LTFE sites included different N fertilization rates depending on LTFE series, treatment, and crops. In this study, we selected field plots with as similar N application rates as possible in order to minimize the influence of N among sites and primarily focused on the comparison between long-term arable farm and livestock farm management systems. However, the N application rates still varied between years and sites (Table 2). Briefly, N application rates ranged from 82 to 100 kg ha<sup>-1</sup> year<sup>-1</sup> for both livestock farm and arable farm management systems at the southern and central sites, and from 30 to 160 kg ha<sup>-1</sup> year<sup>-1</sup> at the northern sites. Moreover, plant residues were incorporated into the soil in arable



**Fig. 1** The locations of 12 long-term field experiments (LTFE) along the latitudinal gradient of Sweden (shown on the map) and the pictures of two sites in Central Sweden (Fors and Klostersgården) for illustration. These 12 sites belong to two series of the LTFE in the Swedish University of Agricultural Sciences, Uppsala (R3-9001 and R8-74B), including 4 and 5 locations from southern and central Sweden, respectively (R3-9001 series), and 3 locations from northern

Sweden (R8-74B series). The series of R3-9001 (“plant nutrition and soil fertility”) aimed to investigate the effects of crop rotation and fertilization rates on crop yield, plant nutrition, and soil organic matter dynamics; the R8-74B series (“Crop production systems in Northern Sweden”) aimed to improve crop rotation system for better yield performance at high latitude regions in Sweden.

**Table 1** Geographical and climatic information, soil clay content, texture and classification from 12 long-term field experiments (LTFE) in Sweden.

LTFE location	Site	Coordinates	Year started	Mean annual		Clay (%)	Texture	Soil classification
				Temperature (°C)	Precipitation (mm)			
Southern	Fjärdingslöv	N55.40233, E13.23217	1957	8.1	590	17	Sandy loam	Haplic Phaeozem
Southern	Borgeby	N55.73566, E13.04675	1957	8.0	569	15	Loam	Eutric Cambisol
Southern	Orupsgården	N55.81986, E13.50589	1957	7.1	777	13	Sandy loam	Haplic Phaeozem
Southern	Ekebo	N55.98917, E12.87401	1957	7.8	683	14	Loam	Haplic Phaeozem
Central	Bjertorp	N58.23518, E13.12812	1966	6.2	571	30	Silty clay loam	Unknown
Central	Klostersgården	N58.47651, E15.50571	1966	6.8	569	50	Silty clay loam	Haplic Phaeozem
Central	Högåsa	N58.50584, E15.44949	1966	6.8	569	10	Loamy sand	Arenic Umbrisol
Central	Kungsängen	N59.83669, E17.68661	1963	5.5	528	56	Clay	Gleyic Cambisol
Central	Fors	N60.31572, E17.50409	1963	5.0	635	18	Silty loam	Calcaric Phaeozem
Northern	Ås	N63.24855, E14.56110	1965	2.9	490	20	Gravelly loam	Podzols
Northern	Röbäcksdalen	N63.80838, E20.23942	1965	3.1	566	10-15	Silty loam	Podzols
Northern	Öjebyn	N65.35816, E21.39188	1966	2.0	500	5-10	Silty loam	Unknown

**Table 2** Crop rotation, application rate of manure, and inorganic fertilizer (nitrogen (N), phosphorus (P), and potassium (K)) of both livestock farm management and arable farm management in two long-term field experiments (LTFE) series (R3-9001 and R8-74B) in Sweden.

LTFE sites		Livestock farm management	Arable farm management
Crop rotation			
Southern		Barley-ley-winter wheat-sugar beet	Barley-linseed-winter wheat-sugar beet
Central		Barley-ley-ley-winter wheat-oat-winter wheat	Barley-oat-linseed-winter wheat-oat-winter wheat
Northern		Barley-ley-ley-pea/oat-potato-ryegrass	Barley-barley-pea/oat or barley-barley-potato
Application rate of manure			
Southern		25 ton ha <sup>-1</sup> every 4th year	n.a.
Central		30 ton ha <sup>-1</sup> every 6th year	n.a.
Northern		20 ton ha <sup>-1</sup> year <sup>-1</sup>	n.a.
Application rate of N, P, and K (kg ha <sup>-1</sup> )			
Southern + Central	N	82–100	82–100
Northern		30–160	30–160
Southern + Central	P	15–40	15–40
Northern		12	12
Southern + Central	K	40–100	40–100
Northern		40–80	40–80

farm management, whereas they were removed in livestock farm management. Overall, four field plots from both livestock farm and arable farm managements were selected at the southern and central sites. At the northern sites, there were three replicates for livestock farm management and four replicates for arable farm management. In total, 93 plots were used in this study.

## 2.2 Soil sampling, storage, and preparation

Due to differences in growing season among sites, soil samples were collected approximately two months after fertilizer applications at each site during 2020. For each plot, 9 to 12 samples from random points within the plot were taken manually, using an auger, from the topsoil (0–20 cm). A composite sample per plot was formed by mixing these samples. All soil samples were mixed thoroughly, kept at the field moisture content and stored in the fridge at ca. 4 °C for up to 1 month until they were transferred to SLU, Uppsala, where all samples were kept in a freezer at –20 °C for up to ca. 3 weeks. Furthermore, subsamples were prepared from each composite sample, including an air-dried subsample for determining basic soil properties; a ground oven-dried sample at 40 °C for both mid-infrared and <sup>13</sup>C solid-state NMR spectroscopy analysis; and a moist subsample which was stored frozen (–20 °C) for soil microbial functional diversity analysis using MicroResp<sup>TM</sup>.

## 2.3 Soil analyses

Total soil C, total organic C, and total N were measured using an elemental analyzer (Trumac CN, Leco Corp, USA).

The soil pH was determined using soil to water ratio of 1:5. Soil clay content was obtained from previous publications in which these soils were analyzed (Kirchmann 1991; Kirchmann et al. 1999, 2005).

### 2.3.1 Mid-infrared spectroscopy analyses

Finely ground oven dried soil was passed through a 250-µm sieve and analyzed using diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) (Alpha II, Bruker GmbH, Karlsruhe, Germany). The measurement was taken at a resolution of 4 cm<sup>-1</sup> and scanned 24 times in the wavenumber range of 4000 to 400 cm<sup>-1</sup>. Reference checks were carried out using the reference golden chip provided by Alpha II instrument (Bruker GmbH, Karlsruhe, Germany). The spectra were determined in triplicate for each soil sample, resulting in 279 spectra. Prior to spectral analysis, values from wavenumbers below 775 cm<sup>-1</sup> were removed, due to measurement noise. Mean values of triplicates, after offsetting the baseline, were then calculated. Subsequently, an adaptive baseline correction was applied to each site using the default coarseness value of 15 in Spectragryph (Spectragryph v1.2.14, Oberstdorf, Germany). The processed spectra, between wavenumbers 4000–775 cm<sup>-1</sup>, were used for further analysis.

### 2.3.2 The <sup>13</sup>C CP/MAS solid-state NMR analyses

A preliminary test run with one sample per each LTFE site was performed in order to screen samples for NMR analysis due to the overall low SOC concentration. The Fors, Fjärdingslöv, Borgeby, and Öjebyn sites all had very low

signal-to-noise ratios and were therefore excluded. Two samples from the livestock farm and arable farm management each from the remaining southern and central sites (6 sites and 24 samples), and all samples for northern sites (Röbäcksdalen and Ås, 14 samples) were analyzed.

Samples were packed into the 4-mm MAS rotors and CP/MAS  $^{13}\text{C}$ -NMR spectra were acquired on a 500 MHz Avance III spectrometer (Bruker BioSpin, Fällanden, Switzerland) equipped with a 4-mm double resonance NMR probe (Bruker, Germany) at 298 K. A spinning rate of 10 kHz was used for all samples, and external chemical shift calibration was performed with adamantane for carbon signals (38.5 and 29.4 ppm). A  $\text{K}^{79}\text{Br}$  reference was used to adjust the magic angle to  $54.7^\circ$ . The experiment consisted of a  $3.0 \mu\text{s}$   $^1\text{H}$  excitation pulse followed by a 1.5-ms cross polarization with a 60.0 kHz spin lock on  $^{13}\text{C}$  and ramped  $^1\text{H}$  spin lock (45–90 kHz). Subsequently, SPINAL-64 sequence during acquisition was used at 83 kHz (Sparrman et al. 2019). Overall, 56 000 transients (time for each measurement was ca. 23 h 30 m) were collected with an acquisition time of 6.8 ms and a relaxation delay of 1.5 s (Sparrman et al. 2019). A line broadening of 200 Hz was applied to all spectra. Manual phasing was performed on all samples but only for zero order, and for the first order, the value calibrated from glycine was used (Taylor 2004). The baseline was manually corrected with only zero-order changes for all experiments.

A blank experiment was run using an empty rotor to ascertain the contribution of the probe contents to the overall signal. This experiment was run under the same conditions except for the number of transients (205,228, measurement time of 3 d 14 h 22 m). Processing of the blank samples was similar to that of the other samples, except for a line broadening of 1000 Hz to decrease the noise of the final subtracted spectrum. Finally, the spectra were obtained by subtracting the blank (scaled to match the difference in the number of transients) from the sample spectra. The assignment of C functional groups was as follows: alkyl (0–50 ppm), methoxyl/N-alkyl (50–60 ppm), O-alkyl (60–93 ppm), di-O-alkyl (93–112 ppm), aromatics (112–140 ppm), O-aromatics (140–165 ppm), and carbonyl (165–190 ppm) (Kögel-Knabner 1997). Signal-to-noise ratios were calculated using the noise region from 380 to 280 ppm and the signal between 80 and 70 ppm. The integration was performed on all spectra (after blank subtraction), and the integral values of different C functional groups were presented as relative proportions (%).

### 2.3.3 Soil microbial functional diversity and functional profiles

Prior to measurement, soil samples were pre-incubated at  $21^\circ\text{C}$  for 2 weeks at a moisture content of 45% of maximum water-holding capacity (MWHC) in order to reactivate

soil microorganisms and stabilize microbial activity. MicroResp<sup>TM</sup> was used to determine the functional diversity (Shannon index) and functional profiles of microbial communities (Campbell et al. 2003). Briefly, 11 substrates including carbohydrates (D-glucose, D-fructose), organic acids (citric acid,  $\alpha$ -ketoglutaric acid), amino acids ( $\gamma$ -aminobutyric acid, L-alanine, L-lysine and L-cysteine-HCl  $\text{H}_2\text{O}$ ), an amino compound (N-acetyl glucosamine), a polymer ( $\alpha$ -cyclodextrin), and an aromatic compound (3,4-Dihydroxybenzoic acid) were used, as these substrates have been shown to discriminate microbial functional diversity (Herrmann et al. 2014). MQ  $\text{H}_2\text{O}$  was used as a blank. After preparing soil samples, 30- $\mu\text{l}$  substrate (or MQ  $\text{H}_2\text{O}$ ) at a concentration of  $30 \text{ mg C ml}^{-1}$  soil water was added to each well, except for the less soluble substrates (L-alanine, N-acetyl glucosamine,  $\alpha$ -cyclodextrin, and 3,4-dihydroxybenzoic acid), for which a concentration of  $7.5 \text{ mg C ml}^{-1}$  soil water was used. All substrates and MQ  $\text{H}_2\text{O}$  were prepared in four replicates on the microplate for each soil sample, and substrate addition brought up soil water content to ca. 63% of MWHC on average. A gel color detector plate was then attached to the plate containing soil and substrate to absorb  $\text{CO}_2$  evolved during the 6h incubation at  $25^\circ\text{C}$ . Soil C- $\text{CO}_2$  was determined by reading the absorbance of the gel plate wells in a plate reader at 570 nm (SpectraMax Plus 384 Microplate Reader, Molecular Devices, USA). A standard curve using exponential function between absorbance and C- $\text{CO}_2$  was prepared for calculating the respired C- $\text{CO}_2$  ( $\text{C-CO}_2(\mu\text{g}) = 0.041 * e^{(8.892 * \text{absorbance})}$ ,  $R^2 = 0.959$ ). The soil samples with pH value  $< 7.0$  were analyzed (70 samples), and alkaline soils were excluded from the analysis due to the reaction with the addition of some substrates, hence the false values in the measurement.

### 2.4 Data processing and statistical analysis

The functional diversity of the soil microbial communities were estimated using the Shannon index ( $H$ ). The MicroResp<sup>TM</sup> profiles were used after subtracting the blank respiration rate from those that had received substrates. The equation below is used for the calculation:

$$H = - \sum_{i=1}^R P_i \times \ln(P_i)$$

where  $R$  is the total number of substrates in MicroResp<sup>TM</sup> analysis and  $P_i$  is the ratio of  $\text{CO}_2$  emissions in response to the substrate  $i$  over the sum of  $\text{CO}_2$  emissions in response to each of the substrates.

Site and treatment effects on SOC, soil TN, soil C/N ratio, and the Shannon index were analyzed using two-way ANOVA, with site and land management as fixed factors, using PAST 4.01 (Hammer et al. 2001). Regression analysis between the relative changes in SOC, TN, and  $H$  was

performed using SPSS 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, USA). Between-class analysis (BCA) of MicroResp™ functional profiles, DRIFT spectra, and the relative proportion of different C functional groups measured by 13C solid-state NMR were performed using the RStudio version 3.6.3 (RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA, 2020). For both ANOVA and regression analysis, a P value of 0.05 was used to indicate the level of significance. With regard to DRIFT spectra, absorbance peaks of (2920 + 2860) cm⁻¹ and (1740 + 1698) cm⁻¹ were selected to represent aliphatic and carboxyl groups, respectively (Gerzabek et al. 2006; Ellerbrock and Kaiser 2005; Artz et al. 2008) and the ratios of absorbance of (1740 + 1698) cm⁻¹/(2920 + 2860) cm⁻¹ were calculated, and the relationship with soil C/N ratio and clay content was analyzed.

Moreover, regression analysis was also used to determine the relationships between geographical factors (latitude, mean annual temperature, and precipitation) and SOC, TN, and H. Regression analysis was made between the natural log transformed respiration rates from MicroResp™ and the nominal oxidation state of C (NOSC) of each substrate for livestock farm management and arable farm management. The values of NOSC were found to be related to the

complete mineralization of specific organic compound, as they are indicative of the energy required to remove electrons during respiration (LaRowe and Van Cappellen 2011). The calculation of the NOSC for each substrate followed LaRowe and Van Cappellen (2011):

$$NOSC = - \left( \frac{(-Z + 4a + b - 3c - 2d + 5e - 2f)}{a} \right) + 4$$

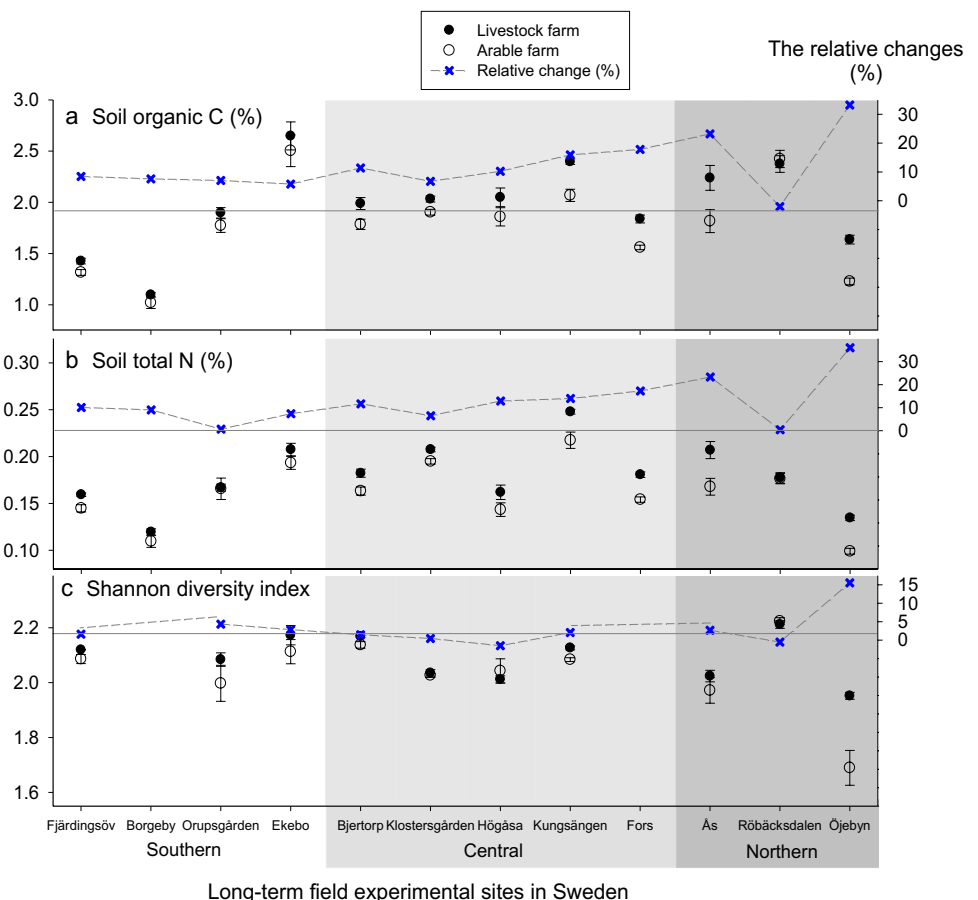
where Z corresponds to the net charge of the organic compound and the letters a, b, c, d, e, and f indicate the stoichiometric numbers of the elements C, H, N, O, P, and S, respectively.

### 3 Results and discussion

#### 3.1 Changes in SOC, total N, and Shannon diversity index under different long-term land management

There were significant differences in SOC contents among LTFE sites (Fig. 2a and Table 3), ranging from 1.0% to 2.6%. After more than 50 years' management, livestock farm

**Fig. 2** Soil organic C (a), total N concentration (b), and the Shannon functional diversity index (H\*) (c) calculated using MicroResp™ measurement in soils from livestock farm management (solid symbols) and arable farm management (empty symbols) from different long-term field experiments (LTFE) in Sweden (Southern sites, no color background; Central, light grey; and Northern sites, gray; sites from left to right correspond to the increases in latitude). The relative changes (%; livestock farm management as relative to arable farm management) are presented on the 2nd Y-axis. The bars represent standard error of the mean where it exceeds the height of the symbol, and in some cases the standard error is too small to be visible. \*Note that only 10 LTFE sites are present in panel, Borgeby and Fors were excluded because the pH was > 7.





**Table 3** Results of statistical analysis on the effect of long-term field experiments (LTFE) sites in Sweden (12) and land managements (livestock farm management and arable farm management) on soil organic C, total N concentration, soil C/N ratio, and Shannon functional diversity index, and also the effect on ratios of absorbance between selected C functional groups ((2920 + 2860) cm<sup>-1</sup>/

(1650 + 1630) cm<sup>-1</sup>, (2920 + 2860) cm<sup>-1</sup>/1515 cm<sup>-1</sup>, (1740 + 1698) cm<sup>-1</sup>/(2920 + 2860) cm<sup>-1</sup>, and the ratio of (1080 + 1050) cm<sup>-1</sup>/(2920 + 2860) cm<sup>-1</sup>) from DRIFT spectroscopy analysis (significant level was indicated as *P* value < 0.05) (\*note that only 10 LTFE sites were included for two-way ANOVA due to high soil pH value (>7) in the sites of Fors and Borgeby).

Soil organic C	Soil total N		C/N ratio		Shannon functional diversity*			
	F value	<i>P</i>	F value	<i>P</i>	F value	<i>P</i>		
Site	72.7	<0.001	60.3	<0.001	191.2	<0.001	25	<0.001
Land management	41.2	<0.001	50.4	<0.001	3.7	0.057	16.4	<0.001
Interaction	1.7	0.084	1.6	0.12	1.4	0.179	3.2	0.004
	(2920 + 2860)cm <sup>-1</sup> /(1650 + 1630)cm <sup>-1</sup>		(2920+2860)cm <sup>-1</sup> /1515cm <sup>-1</sup>		(1740+1698)cm <sup>-1</sup> /(2920+2860)cm <sup>-1</sup>			
Site	F value	<i>P</i>	F value	<i>P</i>	F value	<i>P</i>	F value	<i>P</i>
	275.2	<0.001	53.4	<0.001	203.9	<0.001	203.9	<0.001
Land management	0.4	0.536	0.4	0.536	0.554	0.554	25.0	<0.001
Interaction	2.3	0.019	1.1	0.019	0.390	0.390	1.6	0.114

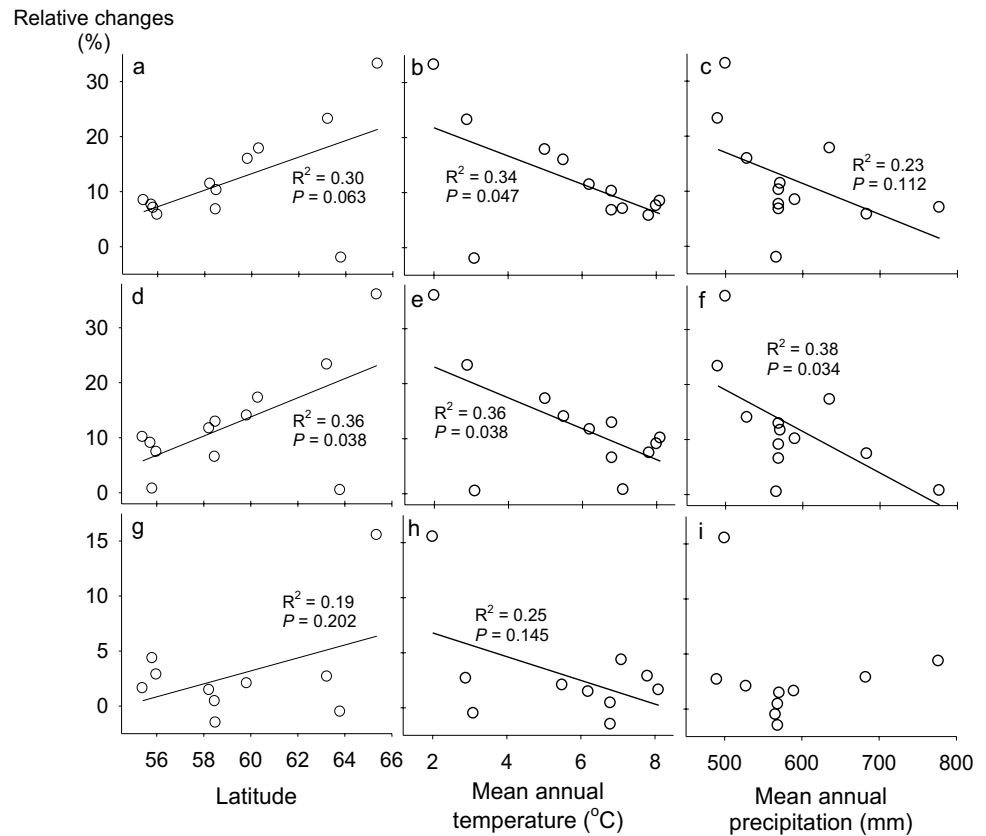
management increased SOC concentrations relative to the arable farm management, with the exception of soils located at the R b cksdalen site (Fig. 2a and Table S1). The relative increase in SOC content ranged from 5.8 % in Ekebo to 33.2% in  jebyn. Similar patterns were found for both total N content and the relative increase in total N (Fig. 2b). The incorporation of ley into crop rotations and organic manure inputs have both been found to be beneficial for soil biophysical properties in many studies (Gerzabek et al. 1997; Martin et al. 2020; van Leeuwen et al. 2015; Zhou et al. 2019), and the combined effect of ley and manure additions is very likely the primary cause for the higher content in soil C and N. This is also supported by a previous study including some of the sites used here, which concluded that the inclusion of ley in crop rotation and manure applications strongly promoted soil ecosystem services (i.e., crop yield), and more importantly, key soil properties (i.e., soil organic C, soil N content, and microbial biomass) were increased (Albizua et al. 2015). In our study, there was a significant and positive relationship between the relative changes (livestock farm vs arable farm management) in SOC and TN as a function of latitude (Fig. 3), with the largest increase in the relative change occurring in the Northern sites (except R b cksdalen), and the smallest increase in the Southern sites. Furthermore, a negative relationship was found between mean annual temperature and the relative changes in SOC and TN (Fig. 3). The impact of latitude and mean annual temperature on both SOC and TN concentration or the relative changes in SOC and TN are consistent with previous studies which found great influence of climatic and geographic factors on the accumulation of SOC (Conant et al. 2011; Dai and Huang 2006). Moreover, there was a significant and

positive relationship in the relative changes between different land management practices in SOC and TN ( $R^2 = 0.93$ ,  $P < 0.001$ ) (Fig. S1a), which reflects the coupled cycling between soil C and N in terrestrial ecosystems (Zinn et al. 2018; Pe uelas et al. 2012).

The functional diversity of the microbial communities, measured as the Shannon diversity index, varied among sites and between land managements, ranging from 1.69 to 2.22 (Fig. 2c and Table 3). With the exception of the Klostersg rden, H g sa, and R b cksdalen sites, livestock farm management resulted in significant increases in microbial functional diversity, with the relative increase ranging between 2.1% and 15.5%. The soil with the lowest functional diversity in arable farm management (1.69,  jebyn) showed the highest relative increase in the Shannon index in the management of livestock farms (15.5%), while soil with the highest index (2.22, R b cksdalen) showed no difference between land managements. Overall, the effect of long-term livestock farm management on the Shannon diversity index was not found in all the LTFE sites, and the impact was not as pronounced as the effects on SOC and total N content. It indicates that the responses in microbial functional diversity are more subjected to changes in local environment and management practices. Thus, our 1st hypothesis (soil organic C content and microbial functional diversity are higher in livestock farm management, compared to the arable farm management) can only be partially confirmed.

Furthermore, unlike the clear influence of altitude and mean annual temperature on SOC and TN content, the Shannon index of microbial functional diversity was not influenced by either factor. It suggests that the microbial functional diversity index has variable responses to both

**Fig. 3** Regression analysis on the influence of latitude, mean annual temperature (°C), and mean annual precipitation (mm) on the relative changes in soil organic C (%) (a, b, and c), on the relative changes in soil total N (%) (d, e, and f) and on the relative changes in Shannon functional diversity index (g, h, and i), respectively.



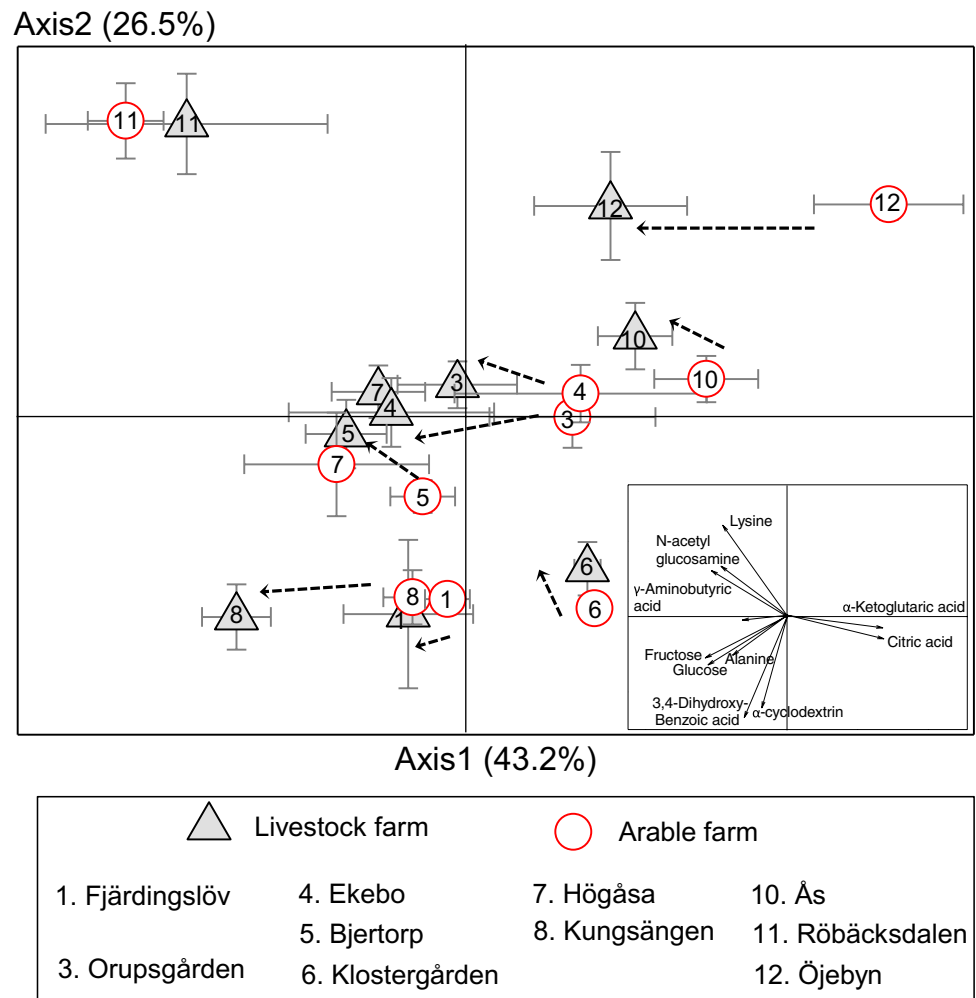
geographical and climatic factors. In contrast to our finding, a study of soils from multiple pine forest locations in Canada found that the microbial functional diversity, measured by the Biolog method, decreased with increasing latitude (Staddon et al. 1998). It strongly suggests that the local environment (soil microhabitat) has an impact in determining the magnitude of the change of microbial functional diversity, reflecting the importance of land management per se. To further confirm this, we calculated the relative changes in the Shannon diversity index (livestock farm management vs arable farm management) and found that there were positive relationships between the relative changes in the diversity index and those in SOC and TN, respectively (Fig. S1). It suggests that the increases in organic C content due to the long-term livestock farm management contribute to the regulation and maintenance of soil microbial functioning (Bongiorno et al. 2020). Similarly, Degens et al. (2000) found higher microbial catabolic diversity in soils with high SOC content. This highlights that the increases in SOC and TN provide positive feedback to the resident microbial communities, possibly related to the input of more diverse substrates to microbial communities in livestock farm management. Overall, local long-term livestock farm management increased SOC and TN content along the latitudinal gradient (except in the case of one of the three Northern sites). Although soil microbial functional diversity

was less responsive to livestock farm management, the relative changes in diversity showed positive correlation with those of both SOC and TN. Hence, long-term livestock farm management represents a sustainable option for enhancing the soil organic C pool and maintaining microbial functioning. In agreement with our results, long-term application of organic matter into soil increased microbial capacity in substrate utilization (Bongiorno et al. 2020). Therefore, the first part of our 2nd hypothesis (changes in microbial functional diversity owing to different long-term land managements will be further influenced by geographical and climatic factors) cannot be confirmed.

### 3.2 Impact of long-term land management on soil microbial functional profiles

Microbial functional profiles were further analyzed to characterize and visualize the impact of either LTFE sites or the long-term livestock farm management on the utilization of specific substrates. Microbial functional profiles showed clear separations along the first two axes of the between-class analysis (BCA), which explained 43.2% and 26.5% of the total variation, respectively (Fig. 4). Separation in functional profiles was found among sites, while the separation between land managements was less pronounced but was observed at the Orupsgården, Bjertorp, Kungsängen,

**Fig. 4** Ordination plot of between-class analysis of soil microbial functional profiles using MicroResp™ in soils from livestock farm management (solid symbols) and arable farm management (empty symbols) from different long-term field experiments (LTFE) in Sweden. The dashed arrows represent the trend from arable farm management toward livestock farm management per LTFE site. The arrows in the inset graph indicate the loadings of different substrates in MicroResp™ measurement. The order of the site corresponds to the increases in latitude. Only 10 LTFE sites are present in the analysis; (2) Borgeby and (9) Fors were excluded because the pH was >7.



Öjebyn, and Ås sites. The samples from the arable farm management taken from Bjertorp and Kungsängen were mainly associated with greater utilization of the polymer ( $\alpha$ -cyclodextrin) and the aromatic compound (3,4-dihydroxybenzoic acid). Microbial utilization of amino acids and carbohydrates was greater in the samples of livestock farm management. The difference between land managements in Ås and Öjebyn were associated with a greater utilization of both amino acids and carbohydrates in livestock farm management. A similar pattern was also found in livestock farm management in Fjärdingslöv, Ekebo, and Klostersgården. Overall, these eight LTFE sites showed consistent patterns in terms of microbial utilization, during which livestock farms generally induced greater utilization of carbohydrates and amino acids. In contrast, greater utilization of carboxylic acids was associated with soils from arable farm management.

Within the eight sites mentioned above, there were subgroups in the ordination plot between LTFE sites (Fig. 4). Fjärdingslöv, Bjertorp, Klostersgården, and Kungsängen were clustered together with greater utilization of both

carbohydrates and amino acids in livestock farm management. This could be due to the addition of manure and the high clay content at the majority of these sites (Table 1). With regard to the effect of the manure application, a broad range of substrates was supplied to the soils by the addition of animal manure (Sonsri et al. 2022). In agreement with this, Bongiorno et al. (2020) also found higher utilization of both carbohydrates and amino acids in farming systems with increased organic matter input, compared to intensive agricultural management with only inorganic fertilizers. This suggests that the presence of easily decomposable substrates in livestock farm management sustained the activities and growth of fast-growing microorganisms. A study by Apostel et al. (2013), which found that soils dominated by fast-growing microbial groups have higher amino acid uptake and incorporation rates, corroborates the results obtained here. With respect to the role of clay content, substrates may interact with soil clay minerals (Sonsri et al. 2022); thus, soils with higher clay content may retain greater quantities of substrates. This may have been particularly true for sites of Bjertorp, Klostersgården, and Kungsängen, which had

the highest clay content of all sites in the study (30, 50, and 56%, respectively, Table 1). This is supported by the finding of a positive relationship between N containing organic compounds and clay and silt content (Grandy et al. 2009). On the other hand, the utilization of polymers ( $\alpha$ -cyclodextrin) and aromatic organic compounds (3,4-dihydroxybenzoic acid) was strongly associated with samples from arable farm management in these sites. It is known that cyclodextrin is a polymer representative of plant materials; hence, it is possible that the higher utilization might be due to the retaining of plant residue in the field (Kirchmann 1991).

Further, the three northern LTFE sites were clearly separated from other sites, in which the sites of Öjebyn and Ås were grouped together. A similar pattern in the utilization of carbohydrate and amino acids was also found in samples from the livestock farm management in Öjebyn and Ås, which is most likely caused by the even higher manure application rate ( $20 \text{ t ha}^{-1} \text{ year}^{-1}$ ) and frequency (annual basis, compared to once every 4 years or 6 years in the Central and Southern sites) in these sites. Besides, the MicroResp results for the soils at Öjebyn and Ås showed greater utilization of both carboxylic acids (citric acid and  $\alpha$ -ketoglutaric acid) in samples from arable farm management, compared to samples from livestock farm management. This suggests that there is a greater recycling of carboxylic acids directly involved in the Krebs cycle in samples from arable farm management (Nunan et al. 2015), suggesting that soil microbial communities have adapted to an environment with a low substrate availability, and obtained the energy for metabolism by recycling intracellular compounds in the Krebs cycle. Further, the Röbbäcksdalen site was separated from all other sites with no differences in the microbial functional profiles between land managements. The historical land use type in Röbbäcksdalen prior to the establishment of the trial is most likely the reason for the observed differences as it was a wetland and had high soil C content initially (between 4.0 and 5.4%). In comparison, the historical land use in other LTFE sites was either arable or grassland. This could explain the clear separation in the response of microbial functional profiles between Röbbäcksdalen and other sites. A previous study observed a decline in SOC content, regardless of land management practices, since the establishment of LTFE in Röbbäcksdalen (Ericson and Mattsson 2000), which is consistent with these results. Generally, in the results from the MicroResp, all samples in Röbbäcksdalen had a strong association with the utilization of amino acids compounds (lysine, N-acetyl glucosamine, and  $\gamma$ -aminobutyric acid). This can be explained by the higher soil C/N ratio (13.4 and 13.7 for livestock farm management and arable farm management, respectively) than all other LTFE sites (Table S1). The direct utilization of amino acids by soil microbial community represents an important strategy for N acquisition (Geisseler and Horwath 2014; Geisseler et al. 2010; Gunina

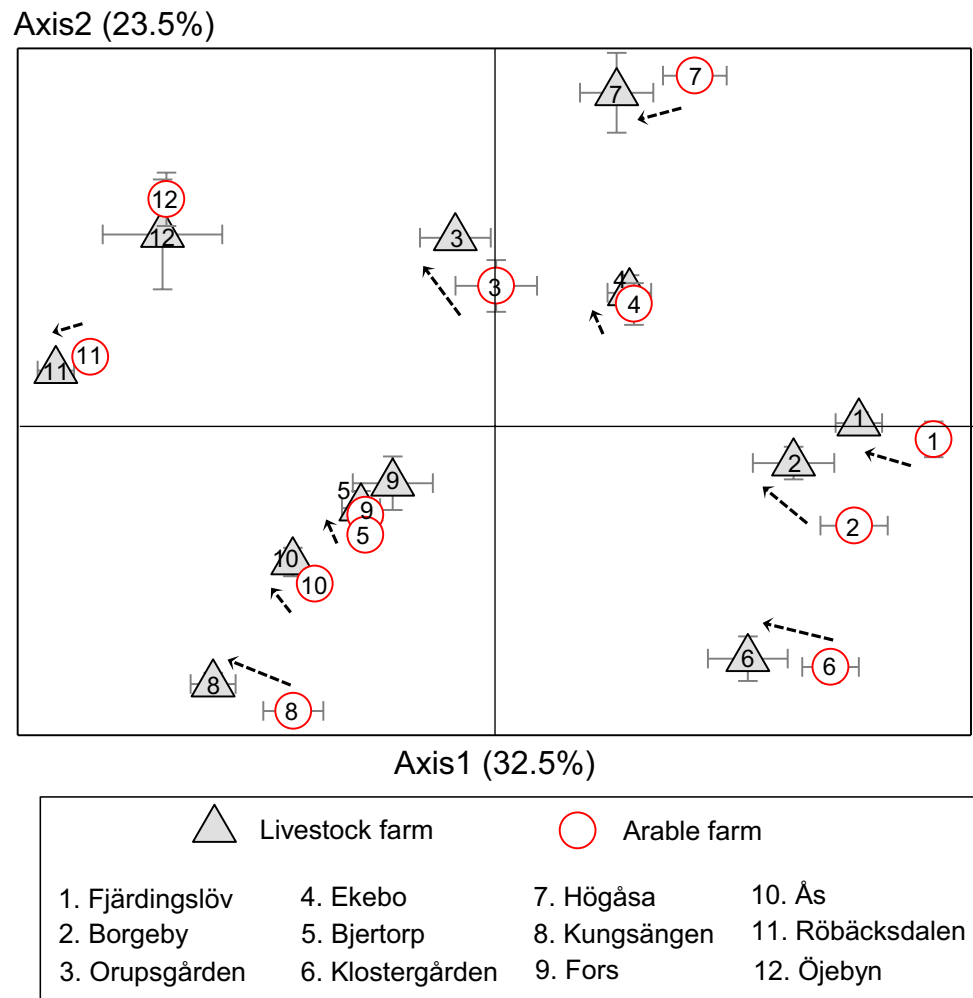
et al. 2014). This suggests that N was limiting at Röbbäcksdalen and microbial communities responded rapidly to the addition of N-bearing compounds. To further confirm the differences between Röbbäcksdalen and other sites, a negative relationship was found between respiration rate and nominal oxidation state of C (NOSC) of 11 substrates in Röbbäcksdalen, whereas the relationship was positive for the other sites (Fig. S2). The negative relationship indicates a preference for compounds with lower NOSC values, e.g.,  $-0.67$  for lysine. Normally, substances with lower NOSC are not preferentially metabolized, because the utilization of such compound requires higher activation energy (Nunan et al. 2015; LaRowe and Van Cappellen 2011). The historical land use type of wetland in Röbbäcksdalen is most likely the cause for the favored utilization of reduced compounds.

### 3.3 Impact of long-term land management on chemical composition of SOC

Our results showed consistent changes in DRIFT spectra between arable farm management and livestock farm management in ten sites out of 12 LTFE sites. Briefly, the BCA ordination graph based on the DRIFT spectra showed separations among LTFE sites and between long-term land managements, explaining 32.5% and 23.5% of the total variation on the axes 1 and 2, respectively (Fig. 5). A clear separation between arable farm management and livestock farm management was visible in six of the LTFE sites: Fjärdingslöv, Borgeby, Orupsgården, Bjertorp, Klostersgården, and Kungsängen in the BCA plot (Fig. 5). Similar, but less pronounced, patterns of change between land managements were also found in the sites of Ekebo, Högåsa, Ås, and Röbbäcksdalen. The absorbance peaks around 1230, 1372, 1428, 1515, 1630, 1650, 1698, 1740, 2135, 2860, and  $2920 \text{ cm}^{-1}$  explained most of the variations. Samples from livestock farm management were more associated with aliphatic C bonds ( $2920$  and  $2860 \text{ cm}^{-1}$ ) and other C functional groups (i.e., the triple bonded C functional groups at  $2135 \text{ cm}^{-1}$ , double bonded C functional groups at  $1740$  and  $1515 \text{ cm}^{-1}$ , and phenolic C-OH at  $1371 \text{ cm}^{-1}$ ), compared to those from arable farm management.

Mid-infrared spectroscopy has been widely used in soil science due to its ease of use, non-destructive nature and high-throughput performance (Le Guillou et al. 2011; Nocita et al. 2015). The consistent changes in DRIFT spectra between land managements suggest the accumulation of C functional groups of similar chemical structure under livestock farms. For example, the absorbance peaks at  $2920$  and  $2860 \text{ cm}^{-1}$  and at  $1650$  and  $1630 \text{ cm}^{-1}$  showed significant interaction between LTFE sites and land management (Table S2). This is consistent with previous studies that the absorbance peaks at  $2920$  and  $2860 \text{ cm}^{-1}$  was proportional to the addition of animal manure or plant litter (Calderón

**Fig. 5** Ordination plot of Between-Class analysis of DRIFT spectra (wavenumber of 4000 to 775  $\text{cm}^{-1}$ ) in soils from livestock farm management (solid symbols) and arable farm management (empty symbols) from different long-term field experiments (LTFE) in Sweden. The dashed arrows represent the trend from arable farm management toward livestock farm management per LTFE site. The order of the site corresponds to the increases in latitude.

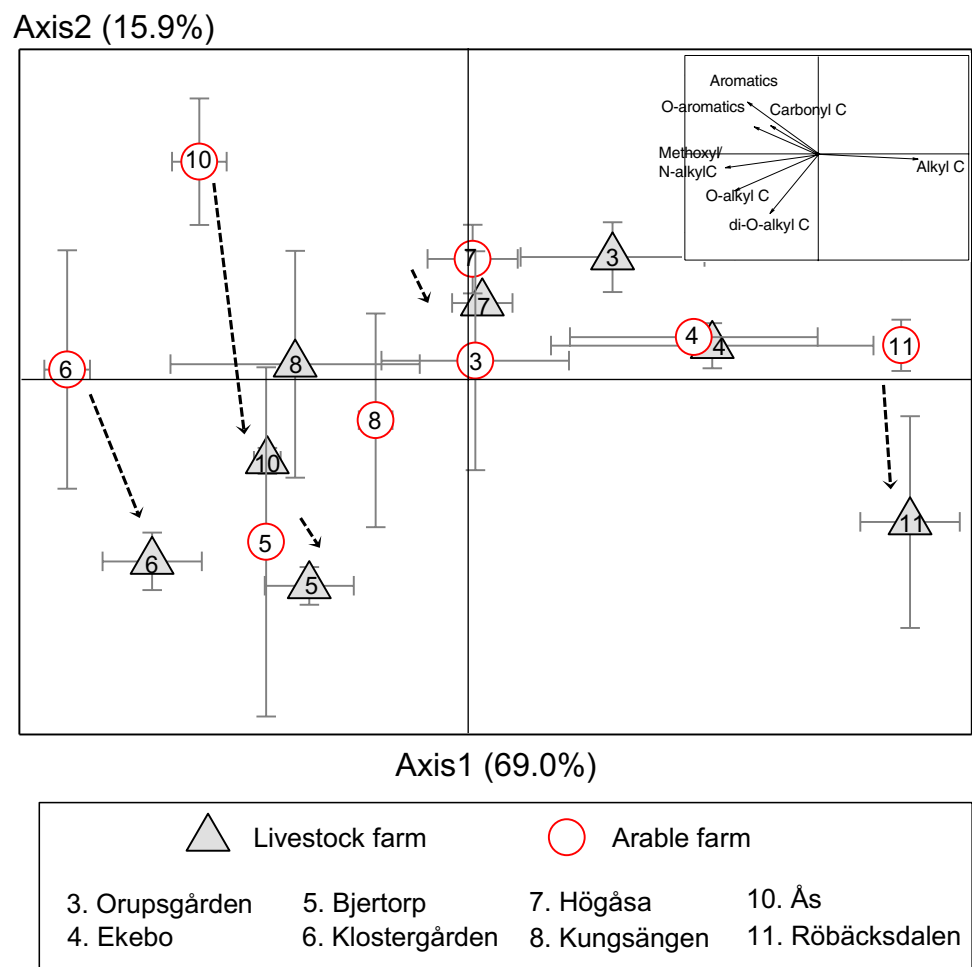


et al. 2011; Voelkner et al. 2015). Moreover, our results showed a positive relationship between SOC concentration and the absorbance intensity at both wavenumbers ( $r = 0.37$ , Table S3), which is in agreement with a previous study by Tremblay and Gagné (2002), and highlights the impact of the long-term manure additions on SOC content. Moreover, livestock farm management was also more associated with absorbance peaks at 1740 and 1698  $\text{cm}^{-1}$ , which represent the markers for easily decomposable substrates for microbial communities (Jones et al. 2003), e.g., carboxylic acids, aldehydes, and ketones (Ellerbrock and Kaiser 2005). Furthermore, the ratio of  $(1740 + 1698) \text{ cm}^{-1} / (2920 + 2860) \text{ cm}^{-1}$  was used as an indicator of substrate availability (Ellerbrock and Kaiser 2005; Artz et al. 2008), which showed positive relationship with clay content (Fig. S3). It suggests that the relative availability of organic acids is increased in soils with higher clay content, compared to the coarse-textured soils (Audette et al. 2021). Given that soil microbial communities utilized organic acids rapidly (Gunina et al. 2014; Jones et al. 2003), it is possible that high clay content soils retain greater amount of organic acids. It could be achieved either

by reducing microbial accessibility to organic acids, or by the association with soil minerals.

To further evaluate the effect of long-term livestock farm management on chemical composition of SOM, the integral values of different C functional groups using the  $^{13}\text{C}$  solid-state CP/MAS NMR spectra (as relative proportion) were analyzed. Overall, alkyl and O-alkyl functional groups were relatively more abundant (ranging from 17.7% to 39.1% for alkyl and from 25.7% to 34.7% for O-alkyl groups, respectively), followed by aromatics (11.5–22.2%) and carbonyl groups (6.7–11.4%). The axis 1 and axis 2 of the BCA explained 69.0% and 15.9% of the total variation (Fig. 6). The differences in chemical composition of SOM between land managements were observed in five out of eight LTFE sites. The most pronounced differences between land managements were found in Klostersgården, Ås, and Röbbäcksdalen (Fig. 6). The arable farm management of these sites showed more abundant aromatics, while soils from livestock farm management were dominated by O-alkyl and di-O-alkyl groups. The sites of Bjertorp and Högåsa showed a similar tendency between the two land

**Fig. 6** Ordination plot of between-class analysis of different C functional groups by  $^{13}\text{C}$  solid-state NMR analysis in soils from livestock farm management (solid symbols) and arable farm management (empty symbols) from different long-term field experiments (LTFE) in Sweden. The dashed arrows represent the trend from arable farm management toward livestock farm management per LTFE site. The arrows in the inset graph indicate the loadings of different C functional groups in  $^{13}\text{C}$  solid-state NMR measurement. The order of the site corresponds to the increases in latitude. Only 8 LTFE sites are present in the analysis; (1) Fjärdingslöv, (2) Borgeby, (9) Fors, and (12) Öjebyn were excluded because of the low signal-to-noise ratios.



managements. Overall, the majority of the soils from livestock farm management were strongly associated with O-alkyl and di-O-alkyl groups, representing the availability of easily decomposable substrates (Kögel-Knabner 2002, Shrestha et al. 2015, Sarker et al. 2018, Sonsri et al. 2022, Mustafa et al. 2022, Demyan et al. 2012). This is probably caused by the long history of cattle manure applications. It was found previously that manure applications supplied large amount of easily available compounds (Mao et al. 2008). This is particularly true at Klostersgården, where the application of manure occurred even before the establishment of the LTFE. In fact, the benefit of long-term application of manure on soil fertility is still visible decades after the last application, and the farm at Klostersgården is still considered to be very fertile (Kirchmann et al. 2005). Additionally, arable farm management was highly correlated with aromatics, which was not clearly shown using DRIFT. The association of aromatics with arable farm management is likely due to the return of crop residues to the soil. The mixing of low-quality crop residues into soil could lead to greater complexity and recalcitrance in SOM composition, compared to the soil with manure addition, which usually

had higher N content (Wander and Traina 1996). Overall, there is a consistent trend with regard to the changes in SOM composition at the majority of the LTFE sites, regardless of the geographical locations and climate conditions. That is, the accumulation of complex aromatics compounds over the long term under arable farm management, while long-term livestock farm management maintains a greater level of easily decomposable compounds, sustaining higher levels of microbial activity. Therefore, the second part of 2nd hypothesis (changes in SOM chemical composition owing to different long-term land managements will be further influenced by geographical and climatic factors) has to be rejected, as the majority of the LTFE sites showed a consistent pattern, as mentioned above.

Further, the sites of Röbbäcksdalen and Ekebo were separated from the other sites and were strongly associated with alkyl C regardless of land management (approximately 40 and 60% higher than the average of other sites, Table S4, Fig. S4). As mentioned previously, Röbbäcksdalen was wetland prior to the establishment of LTFE and hence had the greatest SOC concentration in 1956. Similarly, the association with alkyl C in Ekebo could also be caused by the high SOC content (on

average 2.6%), compared to other sites (1.7%). This suggests the close relationship between alkyl C and SOC concentration, which is in consistent with previous studies (Yao et al. 2019; Mao et al. 2008).

### 3.4 Impact of long-term land management on the interaction between microbial functional diversity and the chemical composition of SOC

Soil microbial communities are the primary driving force in the utilization and transformation of organic matter entering the soil systems, and there may be a linkage between microbial functional diversity and the chemical composition of SOM. Hence, it is likely that the changes in C functional groups would be reflected by the shifts in the pattern of microbial substrate utilization. To test if such linkage exists, a Mantel test was performed between DRIFT spectra and functional diversity profiles measured by MicroResp<sup>TM</sup> (Mantel  $R = 0.24$ ,  $P = 0.001$ ) and between <sup>13</sup>C CP/MAS NMR spectra and functional diversity profiles (Mantel  $R = 0.66$ ,  $P = 0.001$ ). The results confirmed that there were relationships between the changes in soil microbial functional diversity and SOM composition. Although we cannot single out the relative contribution of land management in driving such relationship, the results of the Mantel test suggest a possible relationship between the changes in microbial functional diversity and the corresponding shifts in SOM composition, which merits further investigations in the future. In general, soils from livestock farm management had higher microbial catabolic capacity in utilizing carbohydrates and amino acids. Accordingly, greater accumulation of O-alkyl C and di-O-alkyl C groups was found under the same land management, as suggested by the <sup>13</sup>C solid-state NMR spectra. It was found that the accumulation of these two chemical groups was associated with substrates of relatively reactive and easily decomposable nature (Kögel-Knabner and Rumpel, 2018; Wetterlind et al. 2022). Hence, this may suggest a direct link between soil microbial functioning and SOM chemistry after long-term livestock farm management. Such link is particularly true for the sites of Bjertorp, Klostersgården, and Ås, because they showed very consistent changes in both SOM composition and microbial functional profiles (Fig. 4 and 5). In agreement with our results, higher proportion of reactive C functional groups was found in the manure-amended treatment, compared to the treatment with crop residue addition in a study by Wander and Traina (1996).

## 4 Conclusions

Extensive studies have shown the positive impact of either manure applications or the inclusion of ley in crop rotation on SOM accumulation. Our study suggests that the combination of both practices over the long term (termed as

“livestock farm” management) further enhances soil fertility and microbial activity. We found that the responses of soil organic C and total N content to long-term livestock farm management was significantly related to geographical and climatic factors, while that of microbial functional diversity was less pronounced, indicating the importance of local management practices on microbial functioning. Furthermore, very consistent patterns were found in both microbial functional profiles and SOM composition across soils from different LTFE sites along the large latitudinal gradient. For example, eight out of ten LTFE sites showed similar changes in microbial functional profiles induced by long-term livestock farm management. Similarly, ten out of 12 sites showed consistent shifts in the chemical composition of SOM. Generally, after long-term practices under livestock farm management, soils were associated with a greater utilization of organic compounds in the MicroResp<sup>TM</sup> analysis, e.g., carbohydrates and amino acids. Meanwhile, mid-infrared and <sup>13</sup>C NMR analysis on the same soils showed a high proportion of C functional groups (O-alkyl and di-O-alkyl) associated with more reactive C groups. With regard to the linkage between the changes in microbial functional profiles and the shifts in SOM composition, consistent patterns were found in some LTFE sites (Bjertorp, Klostersgården, and Ås) in both the microbial functional profiles and SOM composition. This highlights an interaction between these two important components of SOM dynamics. Overall, long-term livestock farm management maintained higher levels of soil organic C and N contents, had greater activity and functioning of soil microbial communities, and supplied higher levels of more reactive C functional groups in SOM. This further supports the potential of livestock farm management to improve soil ecosystem services by upregulating both soil biological and chemical properties, thus representing a sustainable option in modern agricultural practices in regions with similar geographical and climatic condition as presented here.

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**Authors' contributions** The research questions were initiated by AH and JW. JW and AS contributed to the detailed experimental design and carried out part of the soil sampling. The majority of the laboratory work was conducted by AS, and JF contributed to the <sup>13</sup>C CP/MAS solid-state NMR measurement and data analysis. NN contributed to data analysis and interpretation. Throughout the preparation

of manuscript, all authors commented on the earlier versions and approved the final draft of the manuscript.

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**Data availability** Data are available from corresponding author upon request.

**Code availability** Not applicable.

## Declarations

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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