

Unraveling the edaphic factors driving organic material decay: Insights from long-term manure application studies

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ABSTRACT

Organic material (OM) decomposition is crucial to soil fertility. We evaluated the hypothesis that long-term manure application induces changes in soil properties which affect the decomposition of OM in the first three months. We buried standardized plant litter with different C/N ratios, i.e., green tea (high-quality OM) and rooibos tea (low-quality OM), in five long-term organic fertilization experiments across different soil types in Europe. Intact 100 cm³ soil cores and bulk soil around the buried OM were analyzed for soil properties, including the physicochemical environment (nutrient contents, pore structure, etc) and microbiological properties (biomass of arbuscular mycorrhizal fungi, saprophytic fungi, actinobacteria, Gram-positive and Gram-negative bacteria, and fluorescein diacetate [FDA] enzyme activity). Despite the difference in microbial growth and activity and soil pore structure between treatments and crops, the effect of manure on OM decomposition was inconsistent across the fields and varied with soil texture and standing crop species. Decomposition of high-quality OM was reduced by 5–7% in two sandy fields with manure treatment and that of low-quality OM was reduced by 22% in one silty manured field, while in the other fields, the decomposition was not affected by manure. The decomposition of both OM types was higher in the maize field than in the barley and grass fields in one sandy site. Clay content and electrical conductivity were negatively linked to the mass loss of both OM types. For the high-quality OM, its decomposition was also negatively linked to soil organic carbon and nutrient content, but positively linked to FDA enzyme activity. In contrast, the decomposition of low-quality OM was positively impacted by the bacterial biomass and soil total porosity. In conclusion, the effect of long-term manure application on OM decomposition depends on the soil texture and the standing crop species, and the edaphic drivers for OM decomposition vary with OM quality.

1. Introduction

Soil organic matter (SOM) is important for soil fertility and sustainable agriculture (Johnston et al., 2009). The formation of SOM is through two main processes, i.e., physical fragmentation of plant litter or other organic materials (OM), and microbial decomposition (Cotrufo et al., 2015). The decomposition rate is affected by several factors including the quality of OM, microbial composition, enzyme activity, soil pore size distribution, etc. (Prescott, 2010; Allison, 2012; Malik et al., 2016; Kravchenko and Guber, 2017). The decomposition of OM is mainly performed by fungi and bacteria, but there is a shift in microbial composition at different decomposition stages (Purahong et al., 2016).

Fungi are the leading decomposer in OM decay, because of their ability to produce extracellular enzymes to break down lignin and other components with high C/N ratios in plant litter (Osono, 2007). Bacteria may utilize the readily available products decomposed by fungal enzymes to degrade complex compounds (Romaní et al., 2006). Unlike saprophytic fungi, arbuscular mycorrhizal fungi (AMF) are obligate biotrophic fungi, but they can also play a role in OM decomposition by influencing the composition of the soil microbial community (Herman et al., 2012; Gui et al., 2017). As fungi and bacteria both take part in the decomposition process and have both synergistic and antagonistic interactions (Romaní et al., 2006), the fungal-bacterial ratio (F:B ratio) is suggested to be important to the carbon cycle. Carbon storage may be enhanced by

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higher F:B ratios (Malik et al., 2016), and variations in the F:B ratio are linked to the aboveground plant species (de Vries et al., 2012).

Soil microorganisms respond to environmental conditions, such as water and oxygen availability (Fierer, 2017), and this availability is primarily controlled by the soil pore size distribution and pore morphology (Kravchenko and Guber, 2017). Kravchenko et al. (2019) reported that pores between 30 μm and 150 μm had higher enzyme activity than pores <30 μm . In the pores <30 μm , the slow-growing *K*-strategic microbes may dominate, and the resources are largely assimilated into microbial biomass, while in the pores between 30 μm and 200 μm , the fast-growing *r*-strategic microbes may predominately act faster and mineralize the SOM, because of better access to water, oxygen, and SOM (Kravchenko et al., 2021). Fukumasu et al. (2022) reported that pores between 0.2 μm and 5 μm were positively correlated to soil organic carbon (SOC), and this is probably caused by the decreased SOM decomposition owing to the limited availability of oxygen. Moreover, microorganisms can release extracellular exudates, which can also serve as a binding agent, for example, glomalin-related soil protein produced from AMF, that forms stable soil aggregates (Wilson et al., 2009). This, in turn, modifies the soil pore network and affects SOM decomposition as the more glomalin-related soil protein there is from AMF, the more pores in the soil (Wilson et al., 2009).

Understanding the mechanism of SOM decomposition allows for managing the soil carbon pool in carbon-depleted systems, especially in the current global warming context (Lal et al., 2015). The SOC content in agricultural fields is usually lower than in their counterpart natural ecosystems, because of the continuous removal of plant biomass and intensive management (Lal, 2004). Amendment methods, including organic fertilizer application, the inclusion of cover crops, and reduced tillage have been proposed and implemented to increase SOC (Johnston et al., 2009; Six and Paustian, 2014). By directly adding OM and nutrients, organic fertilizer (such as manure) application has been shown to increase SOC, enhance microbial biomass and enzyme activity, improve soil aggregate stability, and lower bulk density (Diacono and Montemurro, 2010; Lin et al., 2019). A significant change in SOC normally takes at least 20 years, and thus, long-term experiments (LTEs) in agriculture are vital for monitoring the long-term effect of agricultural practices on soil properties, particularly on SOC (Rasmussen et al., 1998; Diacono and Montemurro, 2010).

Organic material decomposition can be determined by the litterbag method (e.g. Pioli et al., 2020), which typically are mesh bags containing local plant litter and being incubated in a studied biome for a certain period. Keuskamp et al. (2013) proposed to use tea bags as standardized litter bags to study plant litter decomposition on a global scale. In their protocol, green tea and rooibos tea, representing high-quality litter (C/N ratio ~ 12) and low-quality litter (C/N ratio ~ 43), respectively, are buried in soil for about three months in the investigated biomes to study the decomposition rate. It is a simple and convenient method that enables the comparison of litter decomposition in different environments (Djukic et al., 2018). However, studies on litter bags in agricultural systems remain scarce (Chassain et al., 2021). The tea bag study by Fanin et al. (2019a) done in forests suggested that the decomposition of high-quality and low-quality tea depended on climatic and edaphic factors, respectively, whether this finding can be translated into agricultural systems needs further study. Although SOC benefits from manure application, along with other improvements in soil physical and microbiological properties, the influence of manure on litter decomposition is unclear. Some studies found that cattle and poultry manure retarded litter decomposition (e.g. Xi et al., 2023; Weil and Kroontje, 1979) because the added nutrients likely alleviated the nutrient limitation, while others found that farmyard manure enhanced decomposition (e.g. Zaller and Köpke, 2004; Martínez-García et al., 2021) due to the enhanced microbial activity. The study done by Spiegel et al. (2018) also found no significant difference in litter decomposition between cattle manure treatment and control after 56 days of incubation and they attributed it to the well-adapted microorganisms having

similar decomposition manners in LTEs. Thus, the role of manure in OM decomposition still needs further study, and the biotic and abiotic factors involved need to be determined.

Consequently, the main objective of this study was to evaluate the edaphic drivers involved in OM decomposition in soil amended by long-term (>20 yrs) cattle manure application. To achieve this objective, we first investigated the effect of manure on the soil physicochemical environment and microbial properties in LTEs. Second, we investigated OM decomposition in LTEs using the tea bag protocol and assessed the relationship between the fraction of decomposed plant OM (tea mass loss) and the soil physical, chemical, and microbiological properties. We hypothesized that: 1) manure will change soil pore structure to favor the growth of fungi and bacteria; 2) manure will enhance the decomposition of OM, which will be correlated to nutrient content, pore structure, microbial composition and activity.

2. Materials and methods

2.1. Description of sites and sampling

The study involved five LTEs including three fine-textured field sites and two sandy loam field sites. Detailed information regarding the location, field site codes used in this study, start year of each experiment, crops in the field during sampling, and treatment for each site is given in Table 1. At each site, two treatments were selected: unfertilized (UNF) and manured (Manure). The sites are presented in an order of decreasing clay content. The first site had a silty clay texture and was located at the Lanna agricultural research station in Sweden. The site is denoted as SEL hereafter. The treatments were randomized with four blocks with a plot size of 8×14 m. The cropping history during the period 2002–2019 was oat (10 years), spring barley (barley; 4 years), spring wheat (1 year), and winter wheat (wheat; 3 years). Once every second year in the autumn, 8 t ash-free dry matter farmyard manure ha^{-1} (cattle manure with straw bedding) was added to the field before moldboard plowing to a depth of 15 cm (Kätterer et al., 2014). Detailed tillage information about the site can be found in Fu et al. (2022).

The second site had a silt loam texture and is part of the static fertilization experiment in Bad Lauchstädt, Germany. The site is denoted as DEB hereafter. The 4-ha field is divided into eight strips and the size of each plot is 10×26.5 m without field replicates. The two treatments, UNF, and 30 t cattle barn farmyard manure ha^{-1} every second year, were selected from both the barley (strip 2) and maize plots (strip 6). Both plots were rotated with the same crop sequence: barley, silage maize (maize), and wheat. The dry matter of the manure varied from 15% to 46% and the carbon content of the dry matter was around 40%. Both fields were moldboard-plowed to a depth of 28 cm in autumn, and the top 5 cm was tilled in March the following year, before sowing the spring-sown crops. More information and history of fertilization are given by Merbach and Schulz (2013) and detailed tillage information can be found in Fu et al. (2022).

The third LTE is the Broadbalk experiment located at Rothamsted Research, UK and coded as UKB. The 320×150 m field was divided into 20 strips and 10 sections with each strip around 6 m wide since 1968. For this study, the UNF (strip 03) and 35 t fresh cattle-origin farmyard manure $\text{ha}^{-1} \text{yr}^{-1}$ (strip 2.2) in section 2 were selected. The manure contained 248 g of dry matter per kg of fresh manure and the C content in the manure was 364 g per kg of dry matter (Macholdt et al., 2020). The section has been rotated with beans, wheat, wheat, oats, and wheat since 2019. Before this sequence, the 5-year rotation in the section contained oats, forage maize, and three years of wheat from 1997 to 2017 (Macdonald et al., 2019). After having applied manure, the field was plowed in autumn by a tractor-mounted five-furrow reversible plow to a depth of approx. 23 cm (Macdonald et al., 2019). Chalk lime has been applied on this site since the 1950s to maintain soil pH. More information and history of fertilization can be found in the Rothamsted Guide (Macdonald et al., 2019).

Table 1

Description of the experimental sites, weather conditions during tea bag incubation, and total number of tea bag pairs in each site.

Site	Code of the site	Start year	Soil classification ^a	Mean annual temperature (°C) and precipitation (mm)	Crop on site during sampling	Treatment	Carbon and nutrient content of the manure (kg ha ⁻¹)	Last manure application time before sampling	Tea bag incubation period	Mean temperature (°C) and total precipitation (mm) during the tea bag incubation period	Total number of tea bag pairs
Lanna, SE (58°21'N, 13°08'E)	SEL	1996	Aquic Haplocryept ^b	7.3 636 ^b	Wheat	UNF 8 t ha ⁻¹ 2yrs ⁻¹ (dry matter) FYM	C: 4000 N: 241 ^b P, K, Mg content not available	Autumn 2018	May–August 2020	14.7 204.4	24
Bad Lauchstädt, DE (51°24'N, 11°53'E)	DEB	1902	Typic Mollisol	8.7 484 ^c	Barley Maize	UNF 30 t ha ⁻¹ 2yrs ⁻¹ FYM	C: 4000 N: 218 P: 76 K: 284 Mg: 78	Autumn 2018 (barley field) Autumn 2019 (maize field)	April–August 2020	16.4 130.7 ⁱ	16
Broadbalk, UK (51°48'N, 0°22'W)	UKB	1843	Aquic or Typic Paleudalf ^d	10.2 764 ^e	Wheat	UNF 35 t ha ⁻¹ yr ⁻¹ FYM	C:3000 N: 249 P: 49 K: 325 Mg: 34 ^h	Autumn 2020	April–July 2021	12.7 224.6 ^e	10
La Higuera, ES (40°03'N, 04°26'W)	ESL	1985	Typic Haploxeralf	14.6 434.1 ^f	Wheat	UNF 30 t ha ⁻¹ 3yrs ⁻¹ CM	°C:3840 N:288 P: 60 K:276 Mg:96	Autumn 2020	April–July 2021	20.2 38	24
Askov, DK (55°28'N, 09°07'E)	DKA	1894	Typic Hapludalf	7.7 869 ^g	Wheat Maize Barley Grass/clover	UNF 38 t ha ⁻¹ yr ⁻¹ CS	C:800 N:102 P:18 K:93 Mg: not available	Spring 2020 (barley, wheat, maize fields) Spring 2019 (grass field)	April–August 2020	16 227.6	71

UNF, unfertilized; FYM, farmyard manure (from cattle); CM, cattle manure; CS, cattle slurry. The data are from.

^a USDA classification (Soil Survey Staff - NRCS/USDA, 2014).^b Kätterer et al. (2014).^c Eden et al. (2012).^d Blair et al. (2006).^e Sarah et al. (2021).^f Kautz et al. (2006).^g Jensen et al. (2017).^h Macholdt et al. (2020).ⁱ DWD – Deutscher Wetterdienst.[†] Data estimated from Dorado et al. (2003) assuming the dry matter of the manure was 40% of the fresh weight.

The fourth LTE is the international long-term fertilization experiment at La Higuera station, Spain, and it is referred to as ESL hereafter. Every three years, 30 t ha⁻¹ cattle manure containing 320 g kg⁻¹ organic carbon was applied (Dorado et al., 2003). Each plot also received background fertilizer consisting of 26 kg ha⁻¹ of P, as triple superphosphate, and 66 kg ha⁻¹ of K, as K₂SO₄ (Dorado et al., 2003). Three replicates for each treatment were distributed in the field with a plot size of 6 × 8 m each and the field was rotated in parallel with sorghum, barley, and wheat. The plots were plowed by an offset disk plow to approx. 20 cm in July and the grains were drilled in November of the previous year. More information can be found in Dorado et al. (2003) and Kautz et al. (2006).

The fifth LTE is at the Askov research station in Denmark, and it is coded as DKA. The treatments were randomized in four field blocks denoted B2 – B5. The size of each plot in blocks B3 to B5 was 11.68 × 9.40 m, while the plots in the B2 block (grown with wheat) were 7.33 × 9.40 m each. The 37.5 t cattle slurry ha⁻¹ yr⁻¹ applied in the field contained an average of 5% dry matter and the total C in the slurry was approximately 44% (Jensen et al., 2022). The cattle slurry has been applied since 1973, before which the fields were fertilized by farmyard manure. The four parallel blocks were rotated with the same rotation sequence, wheat, maize, barley, and grass/clover mixture (including *Medicago sativa* L., *Trifolium hybridum* L., *Lotus corniculatus* L., *Lolium perenne* L., *Festuca pratensis* Huds. and *Phleum pratense* L., this field is referred to as the grass field hereafter), and all four crops were present in the field in the same year. The under-sown grass/clover mixture in the barley field was left in the field after the harvest of the barley as a rotation element until August of the following year. Therefore, grass/clover was present in both barley and grass fields. The cattle slurry was added in March/April to the surface of wheat plots and before plowing for barley and maize plots. The fields were moldboard plowed to a depth of 18–20 cm in March/April for spring-sown crops and in September for the Autumn-sown crop. Tillage was the same for all treatments for each crop type. The site was limed with Mg-rich lime to maintain soil pH. More information and the history of fertilization are given by Christensen et al. (2019) and Fu et al. (2022).

Thus, there were a total of nine cropped fields at the five LTEs: four fields (barley, maize, wheat, grass/clover) at DKA, two fields (barley, maize) at DEB, and one field each cropped with wheat at SEL, UKB and ESL.

Soil sampling took place from July through August 2020 for the sites in Sweden, Germany, and Denmark, and from July through August 2021 for the sites in the UK and Spain. In each plot, three undisturbed steel soil cores (100 cm³, 6.1 cm in diameter, 3.5 cm in height) and bulk soil were collected from the 5–15 cm soil layer at three different locations in the plot. The samples were taken in four spots in each treatment in UKB. All samples from UKB and the samples except for the bulk soil from DEB were treated as pseudo-replicates due to the lack of field replicates for the analysis of the manure effect on each property. For the analyses that involve tea bags, the pseudo-replicates from UKB and DEB were pooled respectively. The extracted soil cores and bulk soil samples were sealed and stored at 2 °C before laboratory measurements. The soil samples for fatty acid analyses were collected directly into 50 ml centrifuge tubes at the top 5–10 cm from the vicinity of each tea bag burial spot. Thereafter, they were freeze-dried at –20 °C for at least 96 h and kept at –20 °C before the analysis.

2.2. Organic material decomposition

The decomposition of organic material was assessed using the tea bag protocol as described by Keuskamp et al. (2013). Briefly, a pair of Lipton green tea (high-quality OM) and rooibos tea (low-quality OM) were buried at a depth of 8 cm and 15 cm apart. Three to five replicates were buried in each plot at sowing time in April or May (due to the differences in the crop sowing and harvesting time), and retrieved after three months, along with soil sampling. After retrieval, the remaining

tea was extracted from the tea bags, and the plant roots in the tea bags were carefully removed by tweezers. The tea was dried at 60 °C for at least 48 h, and the fraction of tea mass loss was calculated as the mass loss of tea divided by the original mass of tea. The weather data during the tea bag incubation period and total number of tea bag pairs for each site is given in Table 1.

2.3. Soil texture and chemical properties

Basic soil characteristics were measured on air-dried and ground bulk soil (<2 mm). Soil texture was determined by a combined hydrometer and wet sieving method after the removal of soil organic matter with hydrogen peroxide (Gee and Or, 2002). Soil organic carbon (SOC) and total nitrogen (TN) were determined by dry combustion using a Vario MAX cube (Elementar Analysensysteme AG, Langensfeld, Germany). Plant-available potassium (K) and magnesium (Mg) were extracted by ammonium acetate and measured with an atomic absorption spectrophotometer (PerkinElmer Analyst 300, US). Water-extractable phosphorus (P_w) was measured by a modified protocol by Sissin (1971). Briefly, 1 g of soil was pre-wetted with 1 mL of demineralized water for 17 h followed by adding 49 mL of demineralized water and shaking for 1 h at 20 °C. The suspension was then centrifuged at 5000 rpm for 10 min and the supernatant was analyzed for P_w by UV-VIS spectrophotometer (UV-1900, Holm & Halby, Denmark). Soil pH was measured using a pH meter (PHM220, Radiometer Analytical SAS, Lyon) in a soil suspension of 8 cm³ of soil in 30 mL of deionized water. Soil electrical conductivity (EC) was measured using an EC meter (CDM210, Radiometer Analytical SAS, Lyon) in a soil suspension of 4 g of soil in 36 mL of deionized water. The data on the chemical properties from the SEL, DEB, and DKA sites were extracted from a previous paper (Fu et al., 2022).

2.4. Soil pore size distribution and pore structure

Soil pore size distribution was determined by soil water retention with the following procedure. The soil cores were saturated for three days and drained to a matric potential (ψ) of –100 hPa in sandboxes and –500 hPa in a pressure plate apparatus, and the corresponding weight was recorded. After the measurements, the soil cores were oven-dried at 105 °C for 48 h to determine the oven-dried mass. The total porosity (cm³ cm⁻³) was calculated from soil bulk density and particle density. The bulk density (g cm⁻³) was estimated as the ratio of oven-dried mass to the total volume of the soil core. The weight and volume of the stones > 2 mm in the soil cores were deducted in the calculation of bulk density. The soil particle density was estimated based on the clay and SOM content using the model given by Schjønning et al. (2017). Volumetric water content (cm³ cm⁻³) was calculated by multiplying gravimetric water content by bulk density, and the air-filled porosity (e , cm³ cm⁻³) was the difference between total porosity and volumetric water content at a given matric potential.

The equivalent pore diameter (d , μ m) was estimated by $d = -3000/\psi$ (hPa). Here, we divided pore sizes into three classes: 1) pore size $\leq 6 \mu$ m, its volume is equivalent to volumetric water content at $\psi = -500$ hPa; 2) pore size 6 – 30 μ m, its volume equals the difference between volumetric water content at $\psi = -500$ hPa and $\psi = -100$ hPa; 3) pore size >30 μ m, which was considered as noncapillary porosity, was calculated from the difference between total porosity and volumetric water content at $\psi = -100$ hPa.

At field capacity (–100 hPa), the one-chamber non-steady-state method was used to determine soil gas diffusivity (D_p) (Taylor, 1950), with a detailed setup and description given by Schjønning et al. (2013). The soil core was placed in a chamber, with one side connecting to the atmosphere, allowing O₂ in the air to diffuse in the chamber through the soil core. Before the measurements, the chamber was flushed with N₂ and the change in concentration of O₂ in 2 h was recorded. The gas diffusivity of O₂ in free air (D_0) given as 0.205 cm² s⁻¹ was used to scale

D_p for the calculation of relative gas diffusivity (D_p/D_0).

Soil air permeability (μm^2) was measured by the Forchheimer approach with the setup and detailed description given by [Schjøning and Koppelgaard \(2017\)](#). Briefly, compressed air at four pressure values (5, 2, 1, and 0.5 hPa) was applied to the soil core in a chamber with one end open to the atmosphere, and the airflow at each pressure was measured. The Darcian air permeability was calculated from the Forchheimer polynomial regression using the four pressure and flow values.

The soil pore structure was evaluated by tortuosity (T) ([Moldrup et al., 2001](#)) and pore organization (PO) ([Groenevelt et al., 1984](#)) at a matric potential of -100 hPa (indicated in the subscript), i.e. field the capacity, with Equations (1) and (2):

$$T_{100} = \sqrt{\frac{e_{100} D_0}{D_{p,100}}} \quad (1)$$

$$PO_{100} = \frac{k_{a,100}}{e_{100}} \quad (2)$$

where e_{100} , $D_{p,100}/D_0$, $k_{a,100}$, are the air-filled porosity, relative gas diffusivity, and air permeability at a matric potential of -100 hPa (indicated in the subscript), respectively. High pore organization suggests higher pore continuity and fast gas transport while on the other hand, high soil tortuosity suggests a more tortuous pore network and the gas transport may be slowed down or impeded.

The data used for the calculation of T_{100} and PO_{100} and the pore size distribution for SEL, DKA, and DEB sites were extracted from [Fu et al. \(2022\)](#).

2.5. Fatty acid analyses

The protocol for fatty acids analyses was adapted from [Bligh and Dyer \(1959\)](#), [White et al. \(1979\)](#), and [Frostegård et al. \(1991\)](#). Briefly, the freeze-dried soil sample was ball-milled before extraction for 4 min at a frequency of 30 s^{-1} using a mixer mill (MM 400, Retsch, Germany). To keep the samples cool, the cylinders with the soil samples were kept in liquid nitrogen for 5 min before grinding. Around 3 g of finely ground soil sample was mixed with 1.5 mL citrate buffer, 1.9 mL chloroform, 3.75 mL methanol, and 2 mL Bligh & Dyer solution (chloroform:methanol:citrate buffer = 1:2:0.8 (v/v/v)) for lipid extraction. The lipid extract was fractionated on silica acid columns (IsoluteSI, Biotage, Sweden) into neutral lipids and phospholipids by eluting with chloroform and methanol, respectively. The lipids were then methylated by 0.2 M KOH in methanol. The 100 μL methyl nonadecanoic fatty acid (19:0) was added as an internal standard. The analysis was performed using a gas chromatograph (GC Agilent 6890 Series) and the signature fatty acids were identified by the Sherlock Microbial Identification System Software version 4.5.

The soil AMF biomass was measured by the content of neutral lipid fatty acids (NLFA) 16:1 ω 5 ([Olsson et al., 1995](#)). The saprophytic fungal biomass was indicated by PLFA 18:1 ω 9c and 18:2 ω 6,9c ([Kaiser et al., 2010](#)). For Gram-negative bacterial biomass, the signature PLFA were cy17:0, cy19:0 ([Zelles, 1997](#)), and for Gram-positive bacteria, they were i15:0, a15:0, i16:0, i17:0 and a17:0 ([White et al., 1996](#)). For actinobacteria, the signature PLFA were 10Me16:0, 10Me17:0, and 10Me18:0 ([Fierer et al., 2003](#)). The total PLFA was the sum of all the identified fatty acids mentioned above. The F:B ratio was calculated as the saprophytic fungal biomass divided by the sum of Gram-positive and Gram-negative bacterial, and actinobacterial biomass.

2.6. Enzyme activity

The enzyme activity was quantified by the hydrolysis of fluorescein diacetate (3',6'-diacetyl-fluorescein [FDA]) with the protocol given by [Schnürer and Rosswall \(1982\)](#). Briefly, 1.5 g of air-dried soil was mixed with 20 mL sodium phosphate buffer (pH 7.6) and the reaction started

after adding 100 μL 5 mM FDA. After 2 h, the reaction was stopped by adding 3 mL acetone to 3 mL suspension in glass tubes. The mixture was then centrifuged at 3500 rpm for 10 min and the absorbance at 490 nm was recorded. For each plot, two replicates were measured, and the blank was measured for each treatment. As there were no field replicates in the DEB site, three replicates were measured for each treatment.

2.7. Statistics

The statistical analyses were carried out in R software version 4.4.1 ([R Core Team, 2022](#)) and the figures were drawn using "ggplot2" ([Wickham, 2016](#)). Before the analyses, the Shapiro-Wilk test and Levene's test were used to evaluate the normality and the homogeneity of variance, respectively. The data that failed the normality test were transformed to ensure normality. Student's *t*-test was then used to evaluate the difference between UNF and the manure treatment, and the Welch's *t*-test was used for the data with unequal variance. The criterion of significance was $p < 0.05$. The effect of the site on the soil properties and tea bag decomposition was evaluated by linear-mixed effect model (LMM) using the *lmer* function in the *lmerTest* package, with the site as a random effect, and treatment as a fixed effect. The random effect of the crop species was only examined on the dataset from DKA due to the uneven number of crop types in the five sites. The likelihood ratio test was used to test whether the random effect was significant using *exactRLRT* from *RLRsim* package. The relationship between the biomass of different microbial groups and soil pore structural properties was evaluated by Pearson correlation coefficient (*r*) and LMM with each soil pore structural property as a fixed effect and site as a random effect. To explore the correlation between all measured properties, Pearson correlation coefficients were also calculated on the whole data site as well as on separated groups based on the soil texture. A weak correlation was classified as r between ± 0.2 and ± 0.4 , a moderate correlation between ± 0.4 and ± 0.6 , and a strong correlation, $r > \pm 0.6$. Prior to further analyses, the data on soil properties were standardized. To assess the contribution of microbial, chemical, and physical properties to the variance of the dataset, the principal component analysis (PCA) was performed on all sites. To quantify the link between soil properties and tea mass loss, 21 variables including clay and silty content, nutrient contents (SOC, TN, P_w , K, Mg), two chemical properties (pH, and EC), six physical properties (porosity, tortuosity, pore organization, and three pore classes) and six microbiological properties (total PLFA, AMF NLFA, PLFA of fungi, and bacteria, F:B ratio and FDA enzyme activity) were selected by backward stepwise regression with LMM using the *step* function in *lmerTest* with the site as a random effect. The variance inflation factor of the selected variables was examined and the cutoff score of 5 was used for potential variable removal. The root-mean-square deviation (RMSE) was reported to evaluate the multiple linear regression (MLR). For each selected variables, the LMM and individual plots were used for further discussion.

3. Results

3.1. Soil physicochemical properties and pore structure

The silty clay site SEL had around 40 % clay and 46 % silt. The clay/silt content for the silt loam sites DEB and UKB were around 21/67 and 21/56, respectively and that for the sandy loam sites ESL and DKA were 16/21 and 10/20, respectively. Based on the soil texture, the five sites were grouped into sandy/coarse-textured (DKA and ESL), silty soils (DEB, and UKB) and clayey soil (SEL); the latter two soils were also referred to as fine-textured soils. Across the five sites, manure increased SOC regardless of soil texture, and the increment of SOC was the largest in the UKB location (238%), whereas the smallest increase was in the SEL site (21%, [Fig. 1](#)). In general manure increased the nutrients, i.e. TN, P_w , K, and Mg, in the four sites, except for the P_w in the SEL ([Table S1](#)). In contrast, manure had no clear effect on TN, P_w , and Mg in the ESL fields

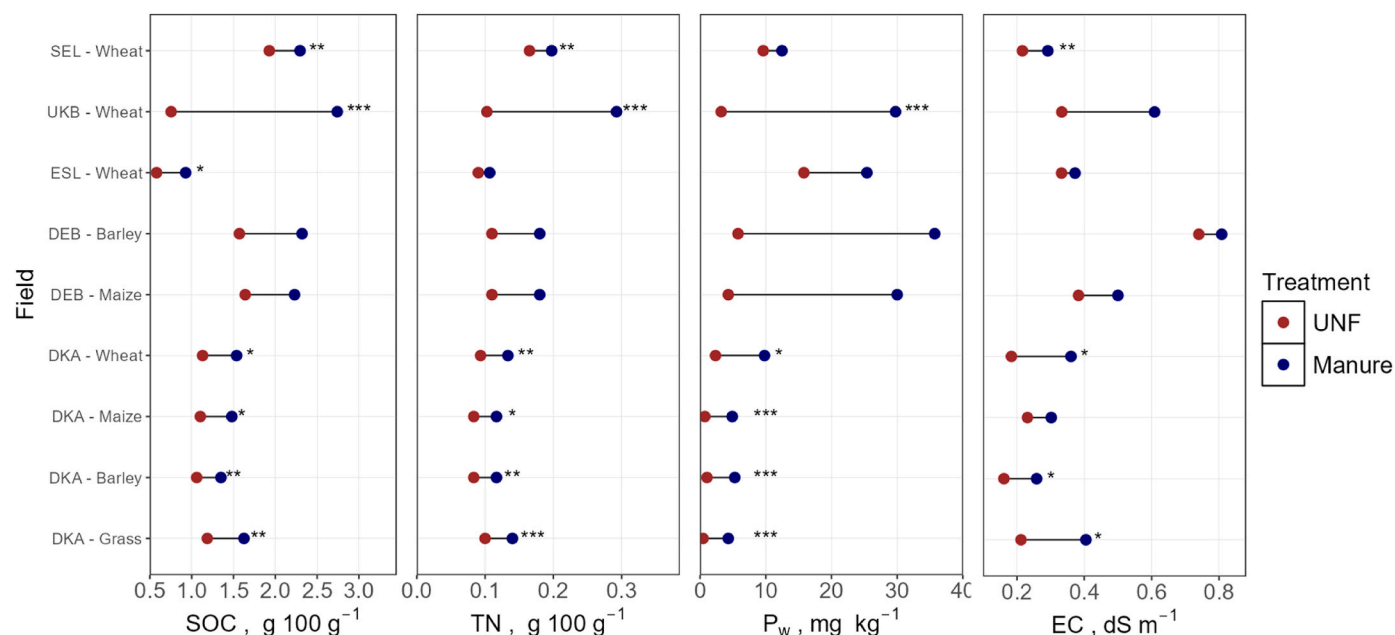


Fig. 1. The content of soil organic carbon (SOC), total nitrogen (TN), and water-extractable phosphorous (P_w), and electrical conductivity between manure treatment and unfertilized (UNF). The crop name after the site code indicates the crop grown in the field during the sampling. The asterisk indicates the significant difference between manure treatment and UNF in the field, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Data from the DEB site were excluded from the statistical analysis. Data from SEL, DEB, and DKA sites were extracted from Fu et al. (2022).

(Fig. 1; Table S1). Nevertheless, the P_w , K, and Mg contents of the ESL site were comparable to its fine-textured counterparts (Fig. 1; Table S1). Except for the treatments, there were also significant differences in SOC, TN, K, and P_w content between the crop species in the DKA site, and the Mg content was not varied with the crop species.

Overall manure increased total porosity ($p < 0.01$). The proportion of three pore size classes changed by manure addition; manure increased the volume of small pores (pores $< 30 \mu\text{m}$) in the fine-textured soils, primarily through the increase in the volume of fine pores (pores $< 6 \mu\text{m}$) by 7–36% (Table S2). About 24%–35% drop in the volume of large pores (pore $> 30 \mu\text{m}$) in the manure-treated fine-textured soils was observed (Table S2). The effect of manure on the coarse-textured soil varied with the crop type present in the field. In the DKA site, manure increased the volume of fine pores only in the barley and grass/clover fields and no significant drop in the volume of large pores was observed. On the contrary, manure did not cause a significant change in the total porosity and pore size distribution in the ESL site.

The pore structure was evaluated by tortuosity and pore organization. At the field capacity, manured fields showed lower tortuosity at the SEL site and DKA wheat field than the UNF, while the DEB barley field with manure treatment had higher values of tortuosity than the UNF (Fig. 2). The significant difference in pore organization between treatments was only observed in the DEB barley field and not in any other fields. Overall, there was no obvious pattern in soil tortuosity or pore organization between treatments ($p > 0.05$).

3.2. Biomass of microbial groups and enzyme activity

Manure showed a tendency to increase total PLFA in eight of nine fields, while the biomass of different microbial groups between treatments varied with soil texture and crops grown in the field (Table 2). In the manured UKB field, the biomass of all microbial groups and FDA enzyme activity in the soil was increased as compared to the UNF soil. In contrast, manure decreased the biomass of all microbial groups in the SEL site, while it showed no effect on the microbial biomass in the ESL site. In the DEB site, manure increased the biomass of bacteria, i.e. actinobacteria, Gram-positive, and Gram-negative bacteria. On the

contrary, manure had no significant effect on the saprophytic fungal biomass in the barley field, and it lowered the biomass of AMF in the same field. In the DKA site, manure increased the biomass of Gram-negative bacteria. In the DKA barley field, manure also increased the biomass of Gram-positive bacteria in the soil. In the DKA fields grown with maize or grass/clover, manure increased the biomass of fungi, and in the grass/clover field, manure also increased the biomass of actinobacteria. Manure also showed a tendency to increase FDA enzyme activity in the DKA site.

3.3. Correlation between microbial biomass and soil pore properties

The relationship between the biomass of microbial groups and pore size classes differentiated between DKA and the other four sites (Fig. 3). Such differentiation between DKA and other sites was also shown in the PCA plot, and the SEL was also separated from the other fine-textured sites (Fig. S1). Therefore, although the ESL site had a coarse soil texture as the DKA site, it was grouped into silty sites. It is worth mentioning that such differentiation did not show in the relationship between the biomass of microbial groups and total porosity (Fig. S2). There were negative correlations between three the microbial groups and the volume of pores $> 30 \mu\text{m}$ when the site were accounted for (Bacteria, $p < 0.01$; Fungi, $p < 0.01$; AMF, $p < 0.001$). On the other hand, there were no clear correlations between the biomass of fungi and bacteria and the volume of pores $6\text{--}30 \mu\text{m}$ for both soil texture groups. Yet, there was a positive correlation between AMF and the volume of pores $6\text{--}30 \mu\text{m}$ when the random effect of the site was considered ($p < 0.05$). The biomass of bacteria and fungi also increased with the increasing volume of pores $< 6 \mu\text{m}$ (bacteria $p < 0.05$; fungi $p < 0.01$). In the clayey site, there was no significant correlation between biomass of microbial groups and soil pore properties.

The correlation between the biomass of microbial groups and soil pore structure varied. The bacteria biomass was positively linked to pore organization ($p < 0.05$). Yet the positive correlation between saprophytic fungi and pore organization was only found in the sandy soil ($r = 0.57$, $p < 0.01$; Table S3). No significant correlation was found in the fine-textured soils. In addition, for the sandy soil, the standing crop

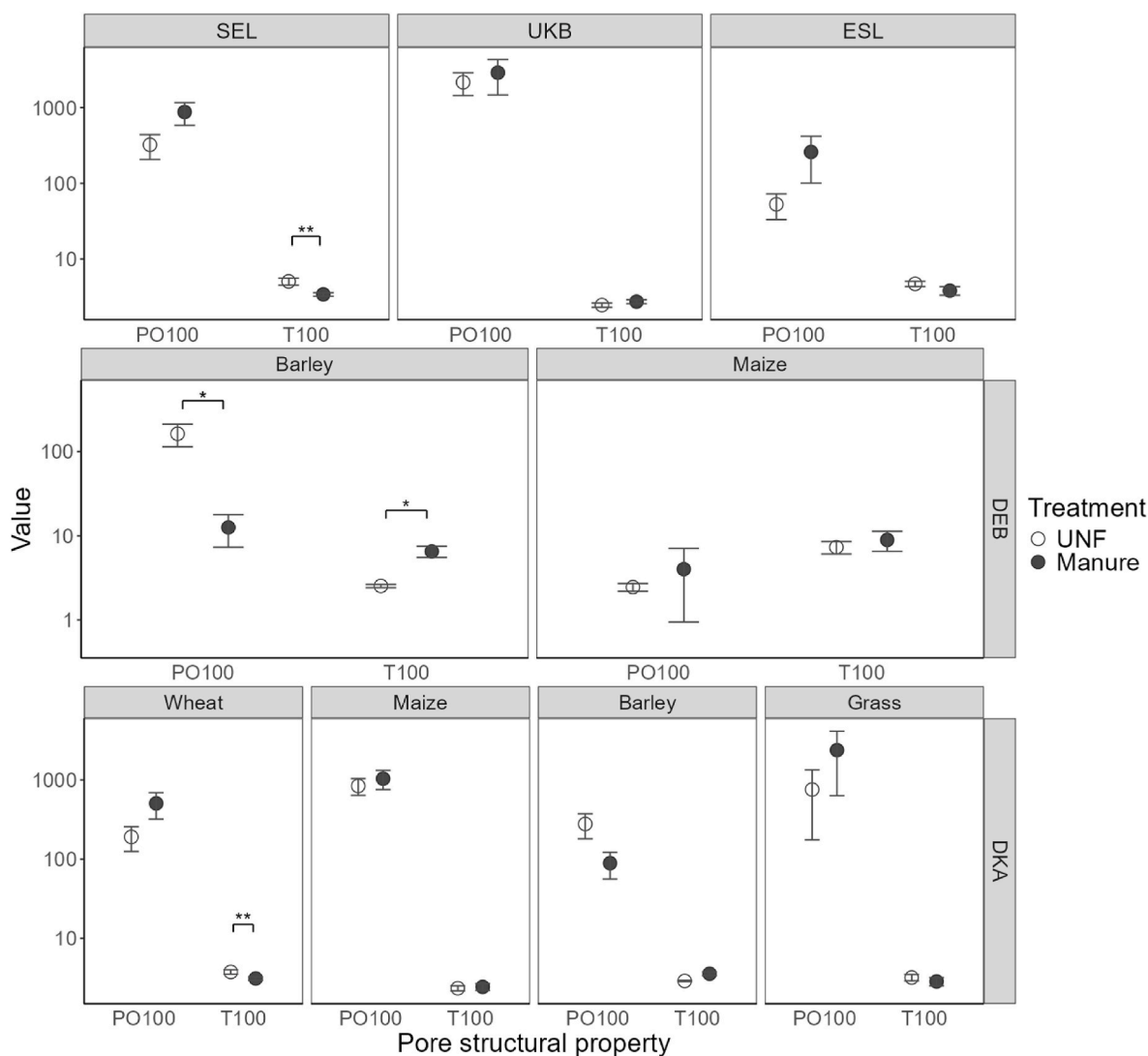


Fig. 2. Soil pore organization (PO100) and tortuosity (T100) at a matric potential of -100hPa under unfertilized (UNF) and manure treatment in the five sites. Data are presented as mean $\pm$ standard error (SE). The asterisk in a certain pore structural property indicates a significant difference between the manure treatment and UNF, *, $p < 0.05$; **, $p < 0.01$. Data from SEL, DEB, and DKA sites extracted from Fu et al. (2022). The analysis for DEB and UKB data was done on pseudoreplicates.

species also had effects on the relationships between soil pore properties and biomass of microbial groups (especially saprophytic fungi and AMF), with grass having the strongest effect.

3.4. Manure effect on organic material decomposition

Considering all fields, 45%–70% of green tea was decomposed, and between 12% and 37% of rooibos tea was decomposed (Fig. 4) after three months. The decomposition of both tea types was the lowest in the SEL site, followed by the ESL site. The difference in the mass loss between the two types of tea was smallest in UKB, around 23% to 25 %, while the largest difference was found in the DEB maize field and the DKA barley field, which was approximately 38%–40% and 35%–42%, respectively. Manure only significantly impacted rooibos tea decomposition in the DEB barley field, and no significant difference was observed in the maize field. In the DKA site, the decomposition of green tea in manure treatment was significantly lower than in the UNF treatment in the wheat and barley fields and the difference was 5% and 7%, respectively. Overall there was no significant difference between the manure treatment and UNF (Green tea, $p = 0.25$; Rooibos tea, $p = 0.39$). For the DKA site, the crop species had an effect on the decomposition. In the barley and grass/clover fields, the mass loss of rooibos tea was

slightly lower than in the other two crops, from 1% to 10%, and showed almost identical values between the two treatments. The grass/clover field also showed a lower mass loss of green tea than the wheat and maize fields.

3.5. Relationship between tea mass loss and soil microbial, chemical, and physical properties

The Pearson correlation coefficients were calculated on the three datasets separately which were primarily based on texture, i.e. clayey (SEL site), silty (UKB, DEB and ESL sites), and sandy (DKA site). The ESL site was grouped into fine-textured soils based on the PCA analysis (Fig. S1). Plant-available Mg were negatively correlated to the mass loss of rooibos tea for both fine-textured soils (Fig. 5), and it was found to be negatively correlated to the mass loss of green tea for the sandy soil. The F:B ratio was negatively correlated to the mass loss of rooibos tea in the clayey soil while it was negatively correlated to the mass loss of green tea in the silty soil. A strong positive correlation between pore organization and mass loss of rooibos tea was found in the silty soil. The content of OC and TN, the volume of pores $>30\text{ }\mu\text{m}$ and all measured microbiological properties had a moderated or strong correlation with the mass loss of green tea for the sandy soil. On the contrary, only three properties

Table 2

Biomass of arbuscular mycorrhizal fungi (AMF), total PLFA, saprophytic fungi, actinobacteria, Gram-positive (Gram+) and Gram-negative (Gram-) bacteria, and fungal:bacterial ratio (F:B ratio), enzyme activity (quantified by fluorescein diacetate analysis, FDA) for the unfertilized (UNF) and manure treatments at the five field sites.

Field	Crop	Treatment	AMF (NLFA)	Total PLFA	Fungi	Actinobacteria	Gram–	Gram+	F:B ratio	FDA
										nmol g ^{–1} soil
										µg fluorescein g ^{–1} soil hr ^{–1}
SEL (SiC)	Wheat	UNF (n = 4)	4.92 ± 0.87	37.09 ± 1.74	7.80 ± 0.36	7.68 ± 0.47	6.60 ± 0.39	14.91 ± 0.81	0.27	31.82 ± 2.28
		Manure (n = 4)	2.96 ± 0.39	23.84 ± 1.53	5.49 ± 0.45	4.78 ± 0.23	5.23 ± 0.29	8.16 ± 0.53	0.3	34.19 ± 1.72
		<i>p-value</i>	0.09	< 0.01	< 0.01	< 0.01	< 0.001	< 0.05	0.06	0.4
DEB (SiL)	Barley	UNF (n = 4)	3.68 ± 1.84	9.93 ± 1.04	2.14 ± 0.18	2.53 ± 0.29	1.34 ± 0.13	3.93 ± 0.45	0.28	6.33
		Manure (n = 4)	0.72 ± 0.13	18.43 ± 3.62	3.15 ± 0.66	5.08 ± 0.93	2.99 ± 0.45	7.19 ± 1.56	0.20	20.80
		<i>p-value</i>	< 0.05	< 0.05	0.2	< 0.05	< 0.05	< 0.05	< 0.01	-
	Maize	UNF (n = 4)	2.56 ± 0.16	10.44 ± 0.5	1.89 ± 0.05	2.87 ± 0.15	1.47 ± 0.15	4.22 ± 0.17	0.22	6.09
		Manure (n = 4)	0.66 ± 0.17	19.13 ± 1.89	3.57 ± 0.42	4.94 ± 0.37	3.59 ± 0.28	7.02 ± 0.83	0.23	15.87
		<i>p-value</i>	< 0.001	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01	0.5	-
UKB (SiL)	Wheat	UNF (n = 4)	0.30 ± 0.07	9.22 ± 1.05	2.03 ± 0.47	1.96 ± 0.30	1.45 ± 0.10	3.78 ± 0.51	0.28	10.28 ± 0.71
		Manure (n = 4)	3.53 ± 0.31	21.14 ± 1.42	4.82 ± 0.56	4.65 ± 0.28	4.44 ± 0.15	7.22 ± 0.64	0.29	30.99 ± 0.91
		<i>p-value</i>	< 0.001	< 0.01	< 0.05	< 0.01	< 0.05	< 0.001	0.9	< 0.001
ESL (SL)	Wheat	UNF (n = 3)	0.92 ± 0.13	15.11 ± 4.38	6.41 ± 2.68	1.13 ± 0.14	2.37 ± 1.83	3.55 ± 0.56	0.88	24.10 ± 1.89
		Manure (n = 3)	0.75 ± 0.30	16.05 ± 1.69	4.79 ± 0.30	1.39 ± 0.24	0.49 ± 0.05	3.69 ± 0.08	0.86	28.05 ± 0.18
		<i>p-value</i>	0.7	0.9	0.7	0.4	0.9	0.5	0.9	0.1
DKA (SL)	Wheat	UNF (n = 3)	2.61 ± 0.60	19.37 ± 0.78	3.59 ± 0.12	3.82 ± 0.10	3.37 ± 0.13	8.58 ± 0.55	0.23	13.79 ± 1.28
		Manure (n = 3)	6.04 ± 1.85	34.92 ± 6.46	7.01 ± 1.76	7.74 ± 1.65	7.12 ± 1.31	12.77 ± 1.61	0.24	24.76 ± 1.42
		<i>p-value</i>	0.2	0.08	0.1	0.08	0.07	< 0.05	0.6	< 0.01
	Maize	UNF (n = 3)	3.14 ± 0.78	24.19 ± 0.47	4.30 ± 0.23	5.95 ± 0.26	4.76 ± 0.04	9.18 ± 0.40	0.22	10.62 ± 1.47
		Manure (n = 3)	2.31 ± 0.43	32.5 ± 3.31	6.25 ± 0.59	7.74 ± 0.84	7.12 ± 0.63	11.28 ± 1.15	0.24	15.53 ± 1.15
		<i>p-value</i>	0.4	0.07	< 0.05	0.1	0.2	< 0.05	0.3	0.06
	Barley	UNF (n = 3)	6.18 ± 1.81	19.79 ± 2.26	3.89 ± 0.76	3.95 ± 0.56	3.19 ± 0.27	8.76 ± 0.76	0.24	13.33 ± 0.68
		Manure (n = 3)	5.21 ± 1.09	24.43 ± 0.41	3.10 ± 0.28	5.25 ± 0.03	4.56 ± 0.07	11.51 ± 0.12	0.15	21.42 ± 0.71
		<i>p-value</i>	0.7	0.1	0.4	0.08	< 0.05	< 0.01	< 0.05	< 0.01
Grass/clover	UNF (n = 3)	21.24 ± 1.35	34.75 ± 1.49	7.81 ± 0.40	7.91 ± 0.35	5.98 ± 0.17	12.32 ± 0.65	0.3	20.30 ± 0.52	
	Manure (n = 2)	18.03 ± 1.63	44.5 ± 1.76	10.08 ± 0.62	9.80 ± 0.03	8.58 ± 0.01	15.38 ± 1.04	0.3	27.63 ± 2.01	
	<i>p-value</i>	0.2	< 0.05	< 0.05	< 0.05	0.08	< 0.01	1	< 0.05	
Fixed effect	(Intercept)	Estimate	2.968	23.709	5.173	4.952	4.426	8.219	0.375	26.442
		SE	1.433	3.743	0.829	0.967	0.873	1.634	0.122	3.555
		t-value	2.072	6.334	6.240	5.122	5.068	5.03	3.065	7.439
		<i>p-value</i>	0.079	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01
	Treatment (UNF)	Estimate	1.053	–5.421	–0.793	–1.377	–1.459	–1.425	0.0119	–8.695
		SE	1.209	1.849	0.531	0.438	0.389	0.632	0.0147	1.376
		t-value	0.870	–2.932	–1.49	–3.143	–3.752	–2.254	0.813	–6.319
		<i>p-value</i>	0.388	< 0.01	0.141	< 0.01	< 0.001	< 0.05	< 0.001	
Random effect	Site	Variance	6.241	59.5	2.575	4.079	3.346	12.108	0.074	57.52
	Residual	Variance	21.883	50.1	4.132	2.813	2.215	5.854	0.003	23.17

SiC, silty clay; SiL, silt loam; SL, sandy loam. Data are presented as mean ± standard error (SE). The SE of the FDA for DEB and the statistical analysis are not available due to the lack of field replicates of air-dried samples.

were showing moderate to strong correlations with the mass loss of rooibos tea with $p < 0.05$. Unlike the mass loss of green tea which was negatively correlated with most properties ($p < 0.05$), the mass loss of rooibos tea was positively correlated to the three soil properties. For the sandy soil, the standing crop species had a significant effect on the relationship between tea mass loss and most edaphic properties except for the relationship between mass loss of green tea and FDA enzyme

activity and volume of large pores.

When all the data were pooled together, the random effect of the site was dropped during the stepwise regression. Because of the high correlation between the bacterial groups (Fig. S3), i.e. Gram-positive and Gram-negative bacteria, and actinobacteria, the sum of the biomass of these three groups was used. The final model showed that the decomposition of green tea was also negatively linked to clay and Mg content,

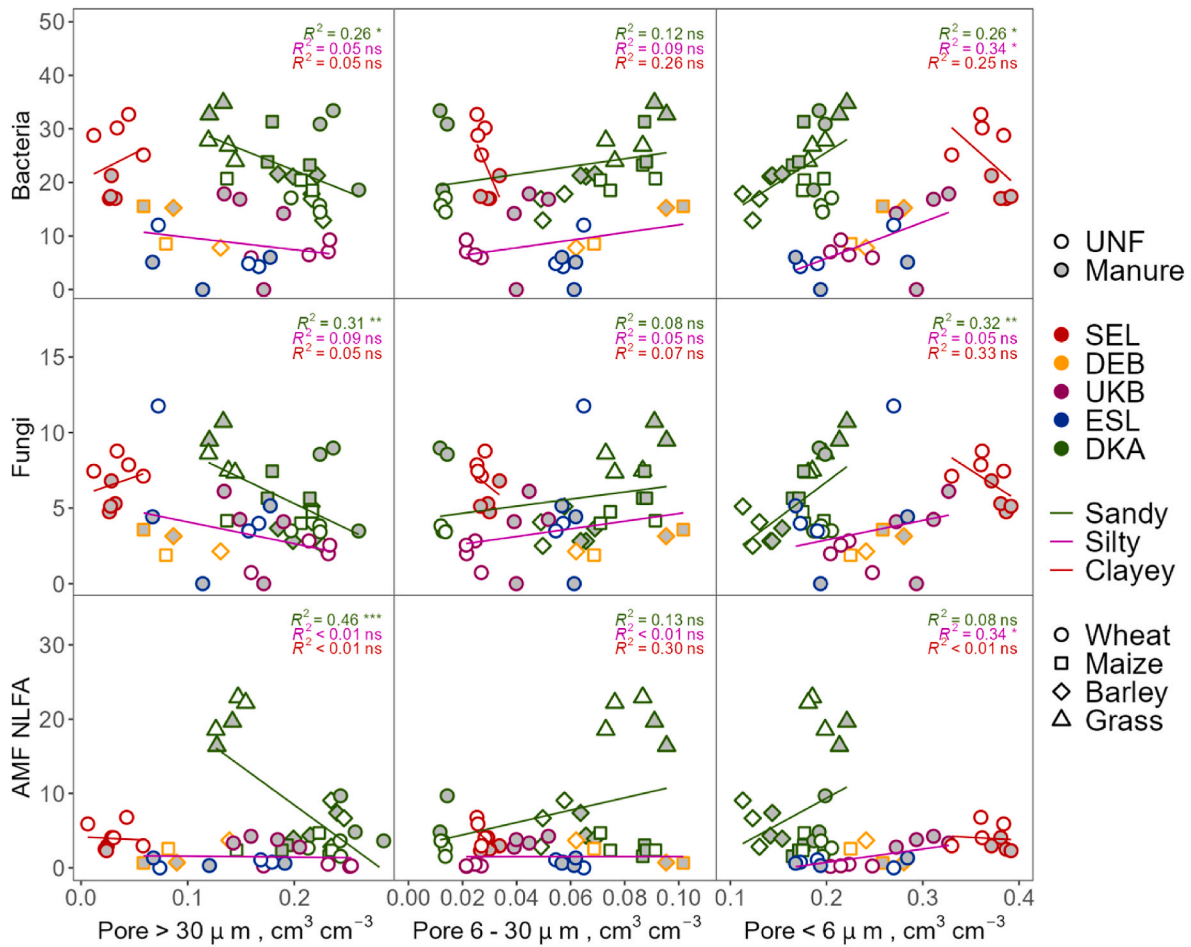


Fig. 3. Relationship between the porosity of three pore size classes and the biomass (nmol g⁻¹ soil) of bacteria, saprophytic fungi, and arbuscular mycorrhizal fungal (AMF, quantified by NLFA) for the five field sites, grouped by soil texture. The lines are the linear regression with R² value for the sandy site (DKA), silty sites (ESL, UKB and DEB), and clayey site (SEL). The asterisks indicated the significance of the regression, ns, not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

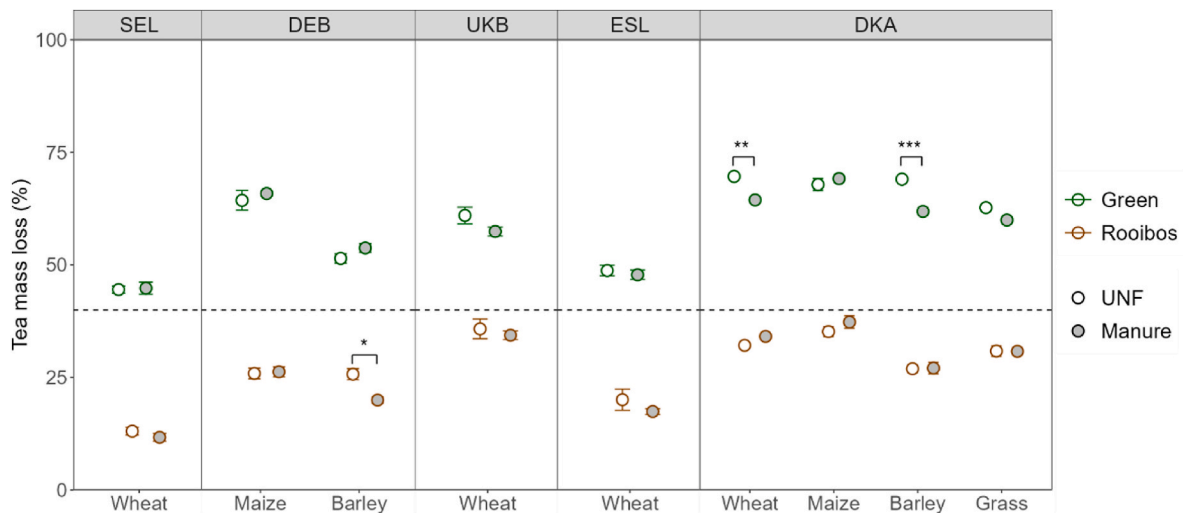


Fig. 4. Tea mass loss of the green tea (above the dashed line) and the rooibos tea (below the dashed line) for the unfertilized (UNF) and manure treatments at the five field sites. Data are presented as mean $\pm$ standard error (SE). The asterisk indicates a significant difference between manure and UNF treatments for the same type of tea, **p* < 0.05, ***p* < 0.01, ****p* < 0.001. Points without asterisks indicate no significant difference. Note that in the DEB and DKA sites, the crops indicated the crops grew in the field during the sampling, and the sampled fields were under the same rotation sequence in each site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

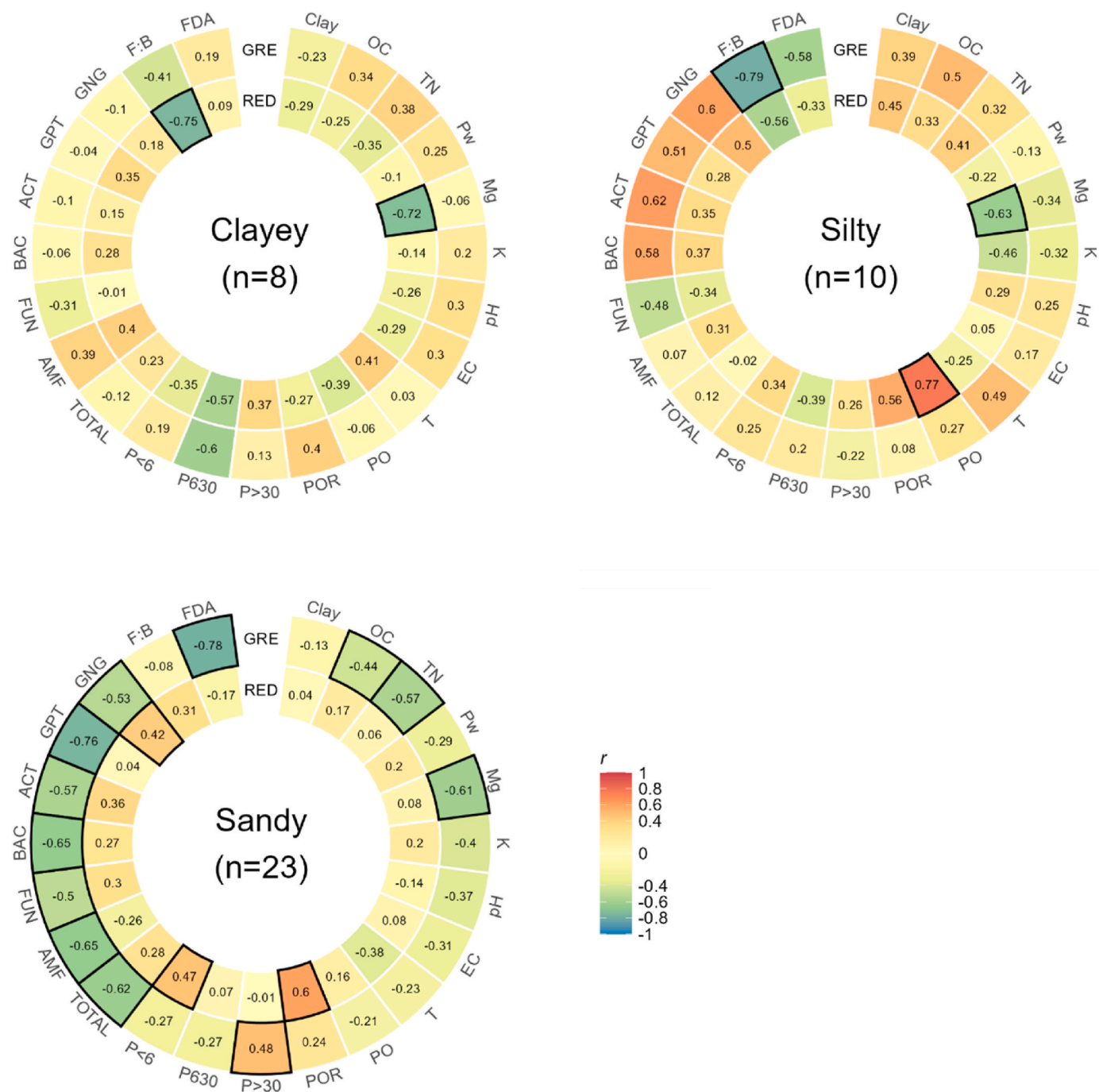


Fig. 5. Pearson correlation coefficients (r) between mass loss of tea and soil clay and nutrient content, chemical, physical and microbiological properties in clayey soils (top left, n = sample size), silty soil (top right, n = sample size), and sandy soils (bottom, n = sample size). The numbers are the correlation coefficients and data with significance level $p < 0.05$ were outlined with a black border. GRE, green tea (high-quality organic material) mass loss; RED, rooibos tea (low-quality organic material) mass loss; OC, organic carbon; TN, total nitrogen; Pw, water-extractable phosphorus; Mg, K, plant-available magnesium and potassium; EC, electrical conductivity; T, PO, tortuosity and pore organization of soil at $\psi = -100$ hPa, respectively; POR, porosity; $P > 30$, the volume of pores >30 μm ; P630, volume of pores 6–30 μm ; P6, volume of pores <6 μm ; TOTAL, total PLFA; AMF, arbuscular mycorrhizal fungal biomass (quantified by NLFA); FUN, saprophytic fungal biomass; BAC, bacterial biomass (the sum of the three bacterial types); ACT, actinobacteria biomass; GPT, Gram-positive bacteria biomass; GNG, Gram-negative bacteria biomass; F:B, fungi: bacteria ratio; FDA, fluorescein diacetate enzyme activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as well as FDA enzyme activity and EC while it was positively correlated to OC content (Fig. 6). The final model for the decomposition of rooibos tea showed that it also negatively linked to clay content and EC, but it was positively correlated to silt, porosity and bacterial biomass (Fig. 6).

4. Discussion

4.1. Manure effect on soil pore structure and microbial biomass

We hypothesized that the manure would enhance soil pore structure, creating conditions more conducive to the growth of fungi and bacteria.

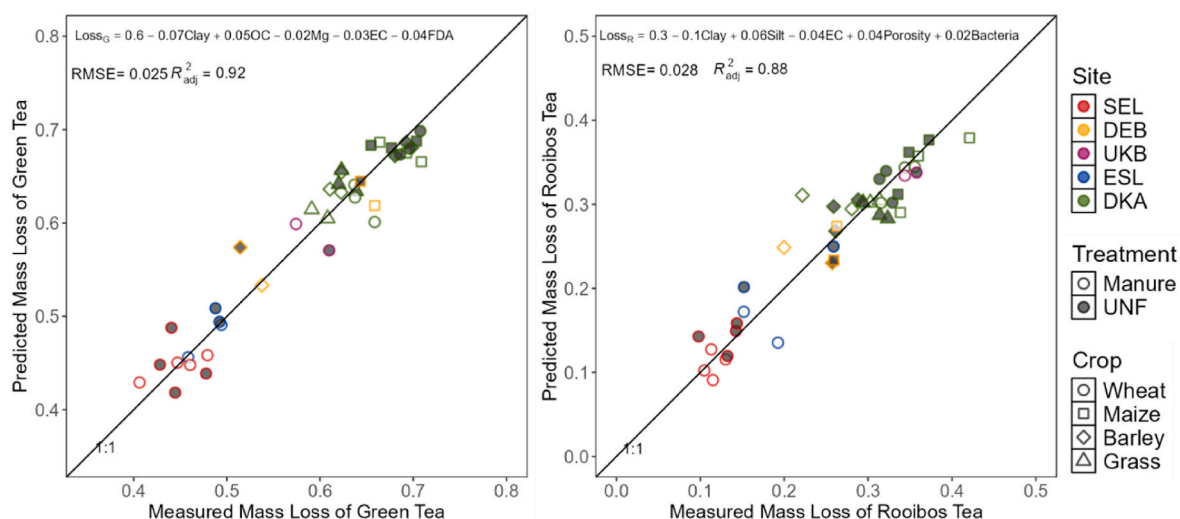


Fig. 6. The predicted mass loss of tea versus measured mass loss for green tea (left) and rooibos tea (right). The equation on the top of each panel is the multiple linear regression equation ($n = 41$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The higher biomass of fungi and bacteria in the soil with more fine pores (pores $< 6 \mu m$) indicated the preference of the microorganisms for fine pores, and partially proved our hypothesis. A higher fraction of fine pores can protect microorganisms, especially bacteria, from predation by protists and nematodes (Erktan et al., 2020). For example, Wright et al. (1995) found that the predation of bacteria by protozoa was much higher in the pores $> 6 \mu m$ compared to the pores $< 6 \mu m$. In addition to the pore size distribution, soil tortuosity and soil pore organization give an insight into pore connectivity by linking air-filled porosity and gas transport (Groenevelt et al., 1984; Moldrup et al., 2001). Well-connected pores would facilitate the air and water flow, in turn, affecting microbial biomass and activity (Kravchenko and Guber, 2017; Tecon and Or, 2017). The results showed that increased soil pore organization rather than soil tortuosity was more likely associated with the manure-treated soil. Soil pore organization evaluates whether the increase in air permeability is caused by an increase in the volume of pores with the same size distribution and continuity (Groenevelt et al., 1984). The higher pore organization value in the manure treatment may suggest more connected pores in the soil matrix in the manured soils. Therefore, the soil at the field capacity (-100 hPa) is expected to be more aerated in the manured fields than in UNF where scattered unconnected pores may be prevalent. Schjønning et al. (2005) presented similar results from the Askov experimental station (coded as DKA in this study) and suggested that manure-treated fields may have a higher degree of continuity of pores compared to the unfertilized fields. The high degree of continuity might be partially attributed to the high earthworm abundance and activity in the manure-treated soil compared to the UNF (Yagüe et al., 2016). The positive correlation between pore organization and biomass of microbial groups in the coarse-textured site suggests that the well-aerated environment possibly caused by manure could enhance the growth of microorganisms. However, such an effect is texture-dependent, and the fungal and AMF growth is not primarily affected by soil pore organization and tortuosity for the fine-textured soils. The effect of soil texture on soil microbial biomass was also reported by Mellado-Vázquez et al. (2019).

In addition, in the sandy soil, the standing crop species also had significant effects on the relationship between the soil pore properties and biomass of bacteria, saprophytic fungi, and AMF, which also indicated the importance of the standing crop species in the field, especially the grass field. Compared to the other eight fields, higher plant diversity may have also attributed to the higher microbial biomass in the grass field (Lange et al., 2015). Moreover, there was no disturbance in the grass field for more than a year, whereas the other fields underwent

tillage four to ten months before sampling. The disruption of physical structure and microbial biomass caused by tillage could also contribute to the difference between the grass field and the cereal fields (Mathew et al., 2012; Crème et al., 2018; Or et al., 2021). Thomopoulos et al. (2023) found that AMF hyphal biomass was largely decreased by plowing compared to reduced tillage, indicating the impact of tillage on microbial growth. Overall, manure-induced changes in soil pore structure may favor the growth of microorganisms, and it also varies with the soil texture and crop species.

Apart from access to water and oxygen, the chemical environment is crucial to microbial growth and composition (Fierer, 2017). Manure increases the content of soil nutrients such as N, P, and K, which is beneficial not only for plants but also for microorganisms (Diacono and Montemurro, 2010). The increase in microbial biomass and enzyme activity by manure has been widely reported (e.g. Ren et al., 2019; Antonious et al., 2020; Chakraborty et al., 2011; Liu et al., 2020), and was confirmed in the present study. Soil with lower sand content and higher SOC favors Gram-negative bacteria and fungi (Hu et al., 2014) and organic N addition could stimulate the growth of fungi and bacteria (Rousk and Bååth, 2007). A significant increase in the biomass of the abovementioned microbial groups by manure addition was thus expected. A high proportion of Gram-negative bacteria could be a sign of more copiotrophic conditions in the soil (Saetre and Bååth, 2000). The fast-growing Gram-negative bacteria may utilize the readily available nutrients for the build-up of biomass and their necromass further facilitates the growth of the slow-growing actinobacteria and Gram-positive bacteria (Fanin et al., 2019b). Between the saprophytic fungi and bacteria, bacteria are considered to be more sensitive to environmental change because the short turnover time of bacteria allows them to react faster to changes in soil management (Lazcano et al., 2013). Accordingly, Wei et al. (2017) reported that a 35-year application of manure decreased the F:B ratio, and therefore, a change in microbial community structure was expected. However, such change was only observed in two fields in this study whereas manure did not affect the F:B ratio in the other seven fields. This can either suggest an uncertain effect of long-term manure on the F:B ratio or question the F:B ratio as a general indicator of microbial changes to environmental changes in agricultural systems.

Various studies have shown organic fertilizers enhance AMF biomass (e.g. Gosling et al., 2010; Yu et al., 2013; Qin et al., 2015). In the present study, manure decreased AMF storage lipids (AMF NLFA) in two fields and increased it in another field, but overall it had no significant effect on AMF. This is partially in contrast to the research by Qin et al. (2015),

who found that pig manure compost increased the biomass of AMF spores. Wang et al. (2009) reported that long-term pig manure addition decreased the spore density and species richness, but increased the abundance of certain AMF species, suggesting that certain AMF species were more sensitive to N. Likewise, Yu et al. (2013) reported that organic fertilizer altered the composition of AMF as the different taxa responded differently to different dosages of the organic fertilizer, which was likely because different AMF species tolerate N differently. Although AMF could compete with saprophytic microbes for inorganic nutrients, the biomass of AMF showed a similar trend as the saprophytic fungi with a volume of large pores ($>30\text{ }\mu\text{m}$). In the DKA site, the particularly high AMF in the grass field in comparison with other cereal crops could also be attributed to the higher plant species richness and the presence of legumes (Jiang et al., 2021). The long soil settling time in the grass field could also contribute to the high AMF biomass as mentioned above. The stronger effect of crop species than fertilization demonstrates the importance of the crop species in the field, which aligned with the findings reported by Stark et al. (2007), where they found that plant type had a larger influence on microbial biomass and enzyme activity than fertilization. Overall, the presence of AMF partially aligned with the presence of saprophytic fungi, and they both negatively affected by the volume of large pores. The saprophytic fungi and bacteria preferred the soil with more fine pores, which were affected by the addition of manure, along with the standing crop species.

4.2. Soil texture and nutrient content drive the decomposition of organic material

Unexpectedly, a non-significant difference in OM decomposition between manure-treated soil and UNF was observed in six out of nine fields, and our hypothesis that manure could enhance the decomposition of OM was thus rejected. Studies by Ochoa-Hueso et al. (2019) further suggested that land use had a much stronger impact on OM decomposition than fertilization, with the latter having an almost negligible effect on tea bag decomposition. Thus, the tea bag protocol may not be a sensitive tool for monitoring the effect of fertilization on soil microbial activity on a farm scale during the plant growing period. Nevertheless, one may still expect that decades-to century-long lack of fertilization may create a highly N-limited environment in the UNF and result in a low decomposition rate compared to the fertilized fields. This finding disagrees with the results reported by Gmach et al. (2024) where they found that Tea Bag Index was sensitive to the agricultural practices including organic amendment in Austria and Sweden. Nevertheless, in three fields in this study, the decomposition of OM was lowered by manure and this is partially in agreement with the results from Xi et al. (2023) and Weil and Kroontje (1979). Spiegel et al. (2018) reported that the OM decomposition rate was significantly lower in the organic fertilizer-treated soil than in the unfertilized soil after 28 days of incubation. However, the same authors also reported that the difference was no longer significant after 56 days because the microorganisms potentially adapted to the different fertilization classes in a long-term experiment. A similar suggestion was given by Middleton et al. (2021), where they reported that mass loss of Rooibos tea after 4-day incubation was more sensitive to the soil health-promoting practices (e.g. + nitrogen, + cover crops) compared to after 130-day incubation. The authors explained that it could be because of greater microbial activity and N availability in the early part of the growing season. Results given by Mori et al. (2023) showed that the decomposition of green tea and rooibos tea was not affected by the nutrient addition in early summer (June to August) in Japan and a clear effect of nutrients on rooibos tea decomposition was observed in midsummer (July to September). This might explain the lack of a clear pattern in our OM decomposition results. A seasonal difference in the tea bag decomposition affected by fertilization is thus expected which needs further investigation.

On a regional scale (i.e. North/West Europe in this study), we hypothesized that the decomposition of OM would correlate to nutrient

content, pore structure, microbial composition and activity. First of all, the quality of OM had an overriding effect on the initial OM decomposition (Zhang et al., 2008), as shown in the difference in mass loss between the high-quality OM and the low-quality OM. A large-scale tea bag study conducted by Fanin et al. (2019a) reported that the decomposition of high-quality and low-quality OM was strongly related to climatic factors and edaphic factors, respectively. Climatic factors were not included in this study because of the unavailable weather data on the microenvironment of each pair of tea bags. In the conceptual model of plant litter decomposition proposed by Prescott (2010), the author suggested that soil moisture and temperature were the primary factors. In this study, the two sites with the lowest tea mass losses could be partially attributed to the low precipitation (ESL) and low temperature (SEL). Yet, soil moisture is also affected by soil texture and soil pore structure. When all sites were considered, soil texture had a strong effect on the mass loss of both OM types. Poeplau et al. (2015) also found that clay content was the major explanatory variable for SOC stabilization after the addition of organic materials. The clay content was strongly correlated to the nutrients, especially Mg, which might cause the negative correlation between Mg and the decomposition of high-quality OM in the MLR. Powers and Salute (2011) found that the Mg addition inhibited the decomposition of leaf litter from tropical forests. The effect of added Mg on litter decomposition is likely associated with enzyme activity and microbial biomass (Giachetti and Vivanco, 2024). It might explain the strong correlation of F:B ratio to the OM decomposition in the fine-textured soils. The different correlation behaviors regarding Mg could be attributed to less leaching in fine-textured soils than in the sandy soil. Moreover, the Mg likely serves as the proxy for the availability of soil nutrients, and the high nutrients could result in less nutrient limitation and lead to the lower decomposition of the introduced OM (tea bags) (Xi et al., 2023). The difference in nutrients is partially the result of the manure addition, and so is the difference in EC. The positive effect of EC was found in the MLR on the decomposition of both OM types, especially for the high-quality OM in the fine-textured soil. It is possibly also because of the higher nutrient availability associated to the higher EC in the manure field than in the UNF. The liming practices may also have affected EC. Overall, the nutrients, especially Mg, played an important role for the decomposition although the effect varied with soil texture and OM type.

Clay content also affects the soil porosity and pore structure, which influences the water and oxygen fluxes and in turn, the decomposition of OM. The positive correlation between soil porosity and OM mass loss, especially the low-quality OM mass loss, suggested the importance of low compaction in facilitating low-quality OM decomposition. Particularly, in the silty soils, the positive role of pore organization on the mass loss of rooibos tea proved that more connected pore path and good aeration was important to the decomposition, especially for soil with finer texture. The higher pore continuity could also help the access of microorganisms to the OM. In the sandy soil, the grass field had lower porosity ($p < 0.01$) and lower mass loss of low-quality OM ($p < 0.05$) than the maize field. The crop density of grass is much higher than maize and the maize field was plowed in the same year of sampling while the grass field was without disturbance for more than a year at the time of sampling. Therefore, the compaction was higher in the grass than in the maize field and the lack of enough oxygen flux might explain the low decomposition, especially for the low-quality OM, in the grass field. Study done by Carlesso et al. (2019) also showed that litter in soil with high bulk density, i.e. low porosity, showed slower decomposition compared to the soil with lower bulk density. Overall, soil physical properties are more important to the decomposition of low-quality OM.

Partially supporting our hypothesis, the microbial community structure (the F:B ratio) had a significant effect on the mass loss but only for the fine-textured soils. As mentioned before, the F:B ratio could be associated with the Mg for the fine-textured soils. For the clayey soil, the low F:B ratio associated with high low-quality OM mass loss suggested the importance of bacteria in breaking down recalcitrant compounds.

This role of bacteria was also found in the other sites as shown in the Pearson correlation and MLR results. Although saprophytic fungi are expected to play an important role in the decomposition of plant OM (Purahong et al., 2016), He et al. (2019) reported that more bacteria was found in litter containing higher lignin content. The positive correlation between bacterial biomass and low-quality OM seemed to be primarily associated with the Gram-negative bacteria. However, it was found that fast-growing Gram-negative tended to use the more available plant-derived OM (Kramer and Gleixner, 2008). Its role on breaking down low-quality OM is thus unclear.

On the other hand, the bacteria, fungi and FDA enzyme activity showed negative correlations with the mass loss of high-quality OM in the sandy site. Moreover, the FDA enzyme activity was a key factor in the decomposition of high-quality OM according to MLR. The negative correlation between FDA enzyme activity and mass loss was unexpected. The tea bag protocol was proposed as a cost-effective tool to evaluate soil microbial activity and it was expected that the more tea decomposed, the higher the microbial activity (Middleton et al., 2021). As an index of microbial activity, high enzyme activity may suggest that the lower mass loss might be attributed to more active microorganisms with high carbon use efficiency, and more compounds were allocated to microbial biomass and products (Cotrufo et al., 2013). Thus, the final mass of tea after incubation should technically be the sum of the remaining tea mass, and the mass of microbial products, microbial necromass, and microbial predators. It can be partially proven by the chemical analysis of the tea done by Duddigan et al. (2020), where the authors showed that the alkyl C associated with microbial metabolites persisted or increased after tea bag incubation. Moreover, the alkyl C was higher in green tea than in rooibos tea before and after incubation, suggesting more microbial metabolites in the green tea after incubation. The high enzyme activity and low OM decomposition might also suggest that manure offered better substrates compared the UNF. Especially, the FDA was more important in predicting the decomposition of high-quality OM than low-quality OM. The readily available compounds from the introduced OM were consumed more by the microorganisms from the poor environment than those from the rich environment. Furthermore, Angst et al. (2021) found lower litter mass loss and higher respiration in clay soil than in sandy soil and it was attributed to the soil texture effect on microbial carbon use efficiency.

Further, the low mass loss of high-quality OM in the grass/clover field might also be because of the root exudates and associated microbial products by the grassroots that penetrated tea bags. The entanglement of green tea leaves with grassroots was more visible and stronger than that of rooibos tea. The presence of fine roots could increase the release of P and K in litter and reduce lignin content in the litter compared to the absence of fine roots (Martins et al., 2021). This could further stimulate the colonization of fast-growing microorganisms. The similar phenomenon was also reported by Martínez-García et al. (2021). Yet, in this study, the boosted microorganisms may prefer the native substrates over introduced OM, which may explain the negative correlation between microbial biomass and high-quality OM decomposition.

In the sandy soil, the decomposition of two OM types showed opposite relationships with microbial properties. Although both types of OM were incubated for the same duration, because of the difference in the quality of the OM, the high-quality OM was likely at the later stage of decomposition at the time of tea bag retrieval, while the low-quality OM was still in the early or middle stage. The role of microorganisms is thus expected to be different in the decomposition of two types of OM. Studies have shown that the quality and quantity of litter can be determinants for the colonization of microorganisms, and thus the microbial communities on green tea and rooibos tea differ from each other and are different from those in the bulk soil (Pioli et al., 2020; Daebeler et al., 2022). Despite the expectation that higher microbial biomass would increase OM decomposition, the increase in the microbial biomass in the sandy soil did not seem to directly translate to the OM decomposition, especially for the high-quality OM. Results reported here

on the correlations between microbial PLFA in the bulk soil and mass loss of OM with contrasting quality suggest the potential preferential choices by microorganisms over plant litters.

4.3. Limitations of the study

It is worth mentioning that other factors could also affect the results. The organic fertilizer application time and the form of fertilizer are different between sites. Ren et al. (2019) suggested that manure type could be one of the predominant factors affecting the response of microbial biomass to manure addition. The DKA site used cattle slurry, and the application time was very close to the tea bag incubation time, unlike in the other sites where cattle manure was applied in autumn every other year or three years. The more readily available N in cattle slurry may have boosted microbial growth and respiration and resulted in higher mass loss than the other sites (Rousk and Bååth, 2007), and it may also explain why the coarse-textured soil in DKA had higher microbial biomass than the fine-textured soils. Moreover, the experimental sites in the present study started in different years. For example, the oldest experiment in UKB had nearly 180 years history, while the experiment in ESL was only about 40 years old. The long history of UKB could explain the drastic difference in soil properties between the UNF and the manure-treated soil.

Although in this study, we focused on the microorganisms, the role of soil meso- and macrofauna in litter decomposition is also important. Studies using litter bags with different mesh sizes showed that meso- and macrofauna contributed significantly to litter decomposition (Weil and Krootje, 1979; Yin et al., 2019). The lack of a clear difference in tea decomposition between treatments may be also ascribed to the lack of fragmentation of litter by soil fauna; the non-woven tea bags prevented the involvement of soil fauna. A larger difference between treatments may be observed when soil fauna is included; however, the inclusion of soil fauna is out of the scope of this study. In addition, we are aware that it would be ideal to sample several times throughout the tea bag incubation period to have a more informative microbiological dataset. However, as the tea bags were buried under crops, sampling near tea bags during the crop-growing period could affect crop growth and yield in the LTEs. Hence, the sampling was done during the harvest time.

5. Conclusion

We investigated how long-term manure application affected organic material (OM) decomposition and the edaphic properties involved in the process for soil with contrasting textures from five LTEs. Apart from the added nutrients, manure likely boosted microbial growth through the modification of the soil pore structure. Manure increased the volume of fine pores in the fine-textured soil and enhanced pore organization in the coarse-textured soil which may favor microbial growth. However, despite the changes in soil properties caused by manure, manure lowered the decomposition of OM in three fields and had no effect in the other fields. In addition, the crop species showed a clear effect on OM decomposition in the sandy site. Overall, the decomposition of both OM types was affected by clay content and electrical conductivity. For the fine-textured soil, plant-available Mg and F:B ratio were the common factors that affected the OM decomposition. For the coarse-textured soil, the edaphic factors that affected the organic matter decomposition varied with OM type. The decomposition of high-quality OM was negatively affected by nutrients and microbiological properties and for the decomposition of low-quality OM, the soil porosity played an important role. In general, for the high-quality OM, soil nutrient availability and enzyme activity were the key factors for its decomposition, and the physical properties were less important. On the contrary, for the low-quality OM, the bacterial biomass and total soil porosity had a stronger impact on the decomposition. The results revealed the contribution of soil physical, chemical, and microbiological properties varied with OM quality, crop species, and soil texture. In addition, this study

also casted doubt on the effectiveness of the tea bag protocol in the evaluation of long-term organic amendment in croplands on a small scale, and the protocol may have to be modified to serve the purpose of smallholder farmers.

CRedit authorship contribution statement

Yuting Fu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabine Ravnskov:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Marcos Paradelo:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Lis W. de Jonge:** Writing – review & editing, Funding acquisition, Conceptualization. **Emmanuel Arthur:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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