Plant Soil (2025) 508:767–784
https://doi.org/10.1007/s11104-024-06829-x

RESEARCH ARTICLE

Salix species and varieties affect the molecular composition and diversity of soil organic matter

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Received: 3 April 2024 / Accepted: 25 June 2024 / Published online: 5 July 2024

Abstract

Background and aims Most studies of the relationships between the composition of soil organic matter and plant cover have been carried out at the plant genera level. However, they have largely overlooked the potential effects that plant varieties, belonging to the same genus, can have on soil organic matter.

Methods We investigated whether plant varieties belonging to different *Salix* species (*S. dasyclados* and *S. viminalis)* impacted the composition of organic matter using mid-infrared spectroscopy and pyrolysis GC/MS. Top-soils were taken from an 18 year-old long-term field trial where six *Salix* varieties were grown as short-rotation coppice under two fertilisation regimes.

Results Significant differences in the molecular composition and diversity of the soil organic matter were observed in the fertilised plots. The effects were mostly visible at the species level, i.e. the organic matter in soil under *S. dasyclados* varieties had higher molecular diversity and lignin content than under *S. viminalis*, potentially due to differences in the amount and composition of their litter inputs. Smaller differences among varieties from the same species were also observed. No significant effects of *Salix* varieties

Responsible Editor: Feike A. Dijkstra.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06829-x.

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were observed in the unfertilised plots. The relatively high degree of spatial variability of several soil properties found in these plots may have masked plant variety and/or species effects.

Conclusion This study provides evidence that the identity of *Salix* species or varieties can affect the molecular composition and diversity of soil organic matter. The corresponding traits should be considered in breeding programmes to enhance soil organic C accumulation and persistence.

Keywords Soil organic matter composition · Molecular diversity · Mid-IR spectroscopy · Pyrolysis GC/MS · Plant varieties · *Salix*

Introduction

There has been a lot of recent interest in soil carbon (C) sequestration (Don et al. 2024) as it has several benefits: (i) the limitation of erosion, (ii) the improvement of water infiltration, purification and storage, (iii) the selection of more diverse and active communities of soil organisms (Rumpel et al. 2022). Additionally, the sequestration of C in soil has become a focus of attention as part of the strategy for mitigating on-going climate change in the context of climate agreements (Kuyper et al. 2018; Panagos et al. 2022).

Changes in several land management practices are known to influence organic C sequestration in soil, but these can be associated with major trade-offs (Amelung et al. 2020). For instance, the conversion of arable land to grassland generally leads to increases in soil organic C, but affects food production (Boysen et al. 2017). In view of these potential trade-offs, the breeding of plant species and varieties with traits related to the quantity and quality of the C input to soil may be a way forward (Poffenbarger et al. 2023; Weih et al. 2014). It has also been shown that the use of management systems with greater interspecific plant diversity can result in, for example, greater aboveground C in forests (Huang et al. 2018; Hulvey et al. 2013) and greater belowground C in grasslands (Hungate et al. 2017; Lange et al. 2015; Prommer et al. 2020). Growing interest exists in developing more diverse agroecosystems (i.e. varietal and/or species mixtures) for agricultural crops (Kopp et al. 2023) and trees (i.e. mixed species stands) (Huuskonen et al. 2021; Stewart et al. 2023). Plant breeding may develop various intraspecific genotypes that enhance the potential to sequester more C in soils. However, there is very little information on the potential effects that varietal diversity or varietal identity might have on soil C dynamics or soil organic C persistence (Semchenko et al. 2021).

It is known that different plant species belonging to the same plant genus produce litter and rhizodeposits that differ in both quantity and composition (Smith 1969; Sun et al. 2017; Warembourg and Estelrich 2001). Furthermore, it has been widely shown that plant–microbe interactions influence both the composition of soil microbial communities (Korenblum et al. 2022; Seitz et al. 2022) and metabolites (Wiesenbauer et al. 2024), and their ability to decompose different forms of organic matter, i.e. their catabolic profiles (Brolsma et al. 2017; Yergeau et al. 2013). However, it is not clear whether these differences result in soil organic matter that differs substantially in quality and/or quantity, especially in the case of the small differences that might be expected across plant varieties (Pérez-Izquierdo et al. 2018).

Changes in the amount of soil C are difficult to detect over short timescales when land use varies (Jandl et al. 2014; Poeplau et al. 2022). The composition of soil organic matter may be more responsive and so may serve as an early indicator of potential changes in organic C persistence and content. In addition to this, it has been argued that the molecular diversity of organic matter may be a driver of C persistence in soil (Lehmann et al. 2020). Furthermore, soil structure is influenced by organic matter composition (Bucka et al. 2019, 2021) and may therefore influence C persistence indirectly, as the physical protection of soil organic C is thought to contribute to organic C persistence (von Lützow et al. 2006). Although the composition of soil organic matter may affect the mechanisms that determine its persistence at the continental scale (Hall et al. 2020b), it is unclear whether the composition of soil organic matter has an important role at the plot-scale where land management systems can change.

The first step is to understand if and how varietal mixtures affect soil C dynamics, i.e. whether there are differences in (i) content and/or (ii) composition of organic matter in soils associated with different varieties. In this study, we chose to work with the plant genus *Salix*. *Salix* is a major woody-perennial bioenergy crop grown as short rotation coppice (Weih

2013). This system is often considered to be a model for studying the relationship between biodiversity and productivity, due to its simplicity and similarity with grassland systems (Weih et al. 2019). Specifically, this system is used here to study how the taxonomic proximity of *Salix* varieties constrain processes related to soil organic C dynamics. The aboveground characteristics of several varieties of the genus *Salix* grown as short rotation coppice in monoculture are well characterized, showing some variation in traits among varieties (Bonosi et al. 2013; Weih and Nordh 2002, 2005). A number of studies have also investigated their belowground traits, such as (i) root biomass (Baum et al. 2018; Hoeber et al. 2017), (ii) fine root composition and decomposition rates (He et al. 2019), (iii) soil and root-associated fungal communities (Baum et al. 2018; Hoeber et al. 2021; Hrynkiewicz et al. 2012; Koczorski et al. 2021), (iv) dehydrogenases activities (Baum et al. 2020), or (v) their impact on the increase of soil organic carbon stock (Baum et al. 2020).

The objective of this study was to determine whether the molecular composition and diversity of soil organic matter was related to *Salix* varieties. Specifically, we hypothesised that different *Salix* plant varieties would lead to soil organic matter with different molecular compositions. The objective was addressed by characterising the composition of organic matter in bulk soil under six different *Salix* varieties in a long-term field trial using two complementary approaches: (i) spectroscopic measurements in the mid-infrared wavelength range (diffuse reflectance infrared Fourier transform (DRIFT)), and (ii) analytical pyrolysis combined with gas-chromatography-mass spectrometry (Pyrolysis-GC/MS).

Materials and methods

Long-term experimental field trial

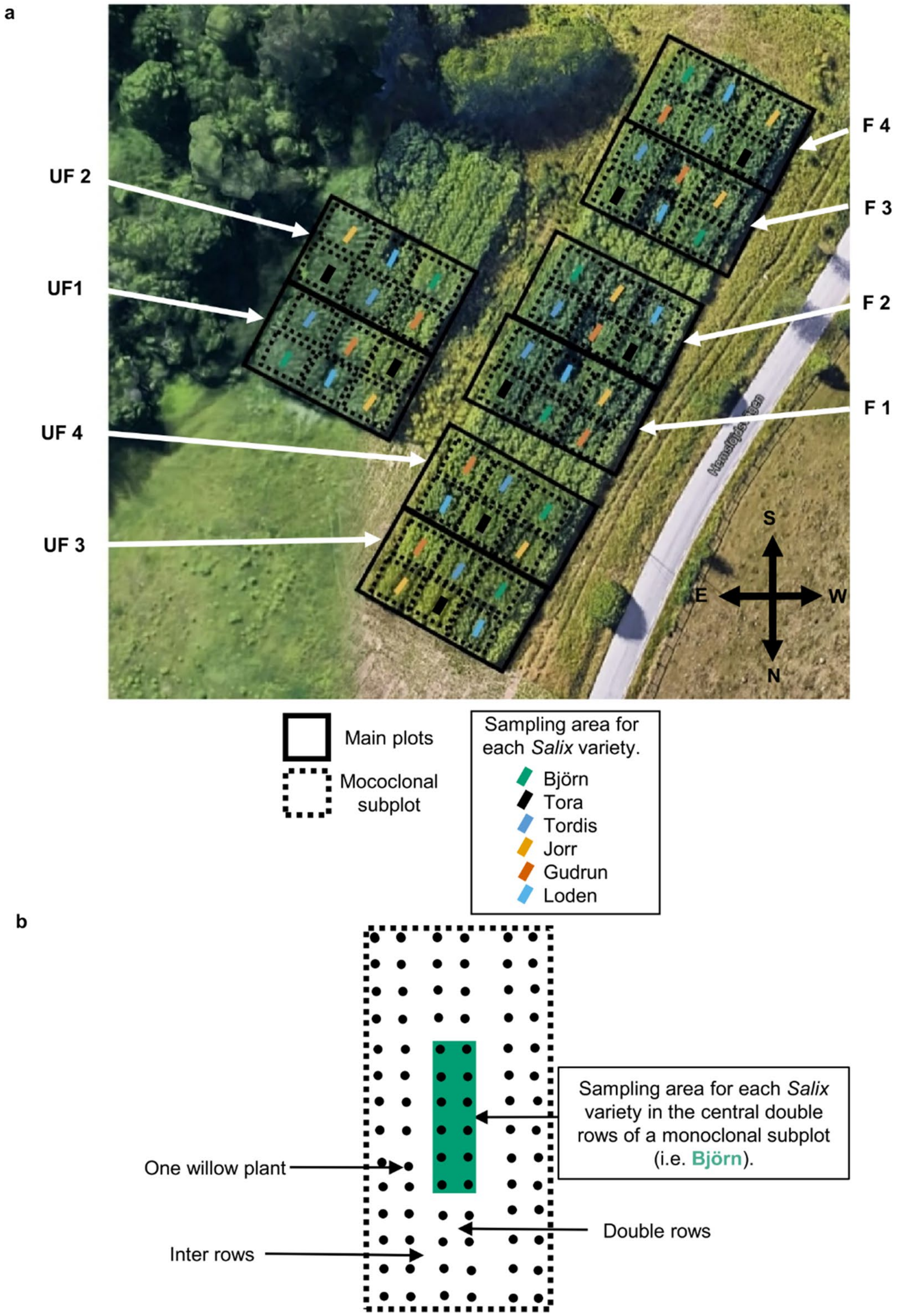
We used a field trial located in central Sweden (59°48′22″N 17°40′24″E). Within this field trial, willow varieties (*Salix* spp.), across which taxonomic distance varied, were cultivated as short rotation coppice on a former arable cropland. The climate is temperate oceanic, and the soil is classified as a Vertic Cambisol, with a texture of 66% sand, 16% silt and 18% clay (Kalita et al. 2021; Weih and Nordh 2005). The field trial was set up in 2001 in a split plot design, with fertilisation as the main plots and willow varieties in subplots. Four of the eight main plots were fertilised annually with approximately 100 kg nitrogen, 14 kg phosphorus and 47 kg potassium per hectare, and the remaining plots were left unfertilised (Baum et al. 2020). Within each main plot, six monoclonal subplots were randomly distributed (Fig. 1a). The subplots measured 6.75 × 7 m and contained 3 double rows of plants and 84 plants in total (equivalent to a density of ~ 18,000 plants ha^{-1}) (Fig. 1b). Each monoclonal subplot contained one of six commercial *Salix* varieties that were more or less taxonomically similar, from full-siblings to differential species (Table 1). Two of the varieties, Loden and Gudrun, are taxonomically close. They have in common morphological traits of the species *S. dasyclados* and are separated taxonomically at the species level from the four others varieties Björn, Tora, Tordis and Jorr that share traits with the species *S. viminalis*.

Sampling strategy

Soil samples were collected from the centre of each monoclonal subplot in April 2019, 18 years after the initiation of the long-term field trial (Fig. 1b). Eighteen sub-samples were taken with an auger (38 mm diameter) from the surface 20 cm, after removal of the litter layer, in an area of approximately 2 m^2. The eighteen sub-samples were mixed to form one composite sample per monoclonal subplot, then sieved (< 4 mm), air-dried and milled. In total, there were 48 composite samples (6 monoclonal subplots × 4 main plots × 2 fertilisation treatments).

General soil properties

Soil pH in each monoclonal subplot was determined using a pH Meter (Radiometer Copenhagen) in a soil:deionised water (1:5 soil:water) mixture at room temperature (23 °C). The total N and organic C contents of the soils were determined by dry combustion using an elemental analyser (TruMac ® CN, Leco corp, S:t Joseph, MI, USA). The total C and total inorganic C contents were determined in two separate runs, and total organic C content (%) was calculated as the difference between total C and total inorganic C content. Total inorganic C content was determined by removing organic matter prior to analysis. This



was achieved by heating the samples to 550 °C for four hours.

Spectroscopic measurements

◂**Fig. 1** Overview of the long-term *Salix* experimental field trial established in 2001 at Pustnäs, near Uppsala, in central Sweden, where six varieties of *Salix* were cultivated as short rotation coppice. **a** Design of the field trial: unfertilised (UF) and fertilised (F) treatments. **b** Representation of one monoclonal subplot, Björn, with sampling area indicated in green, individual willow plants are indicated as dots

Mid-infrared spectra (mid-IR) were recorded in quadruplicate using dry, milled soil, resulting in a total of 192 spectra (48 samples × 4 analytical replicates). The samples were scanned using a Fourier transform IR (FT-IR) Alpha II Drift spectrometer (Bruker, Germany, Serial No. 12547393) equipped with a diffuse reflection (DRIFT) module. We used a spectral resolution of 4 cm^{-1}. Background measurements were carried out using a gold standard. Each spectrum was the average of 24 scans. The apparent absorbance (A) was determined from the reflectance (R) spectra ($A = \log 1/R$) (Guillou et al. 2015). The signal of the spectra between 630 cm^{-1} and 400 cm^{-1} was very noisy and removed from further analyses. The spectra, therefore, contained data from 4000 cm^{-1} to 630 cm^{-1} (2500 nm to 15,873 nm). Each spectrum was pre-processed using the Savitzky-Golay functions of the R package "prospectr" (https://CRAN.R-project.org/package=prospectr) with a first derivative using three point smoothing and a second order polynomial (Savitzky and Golay 1964). Standard normal variate was applied to correct the light scattering (Morais et al. 2020). The mean of the apparent absorbance of four mid-IR replicates was determined for each sample using the summarise function in the R package "dplyr" (version 1.8.6) (Wickham et al. 2019). The full mid-IR spectral range was used for multivariate statistics (Ramírez et al. 2021). Regions of the mid-IR spectra were then assigned to molecular functional groups based on previous studies (Parikh et al. 2014; Sharma et al. 2021). Although minerals can affect the mid-IR spectra, it was felt that this was not a problem as the plots in the present study were small (see above) and therefore likely to have a fairly homogeneous mineral content.

Pyrolysis – gas chromatography – mass spectrometry (Pyrolysis-GC/MS)

Soil samples were analysed by Pyrolysis-GC/MS in the presence of tetramethylammonium hydroxide (TMAH) (25% methanol), an alkylating agent that improves the detection of polar compounds. Around 6 mg of sample was loaded in a quartz tube with 10 µl of TMAH. Glass wool was placed on top of the sample and the sample was then pyrolysed (Pyroprobe 6250, CDS) at 650 °C for 15 s. The pyrolysis products were transferred via a transfer line, maintained at 300 °C, to the injector of a gas chromatograph (7890B, Agilent) operated in split mode (20 ml/min). The separation of pyrolysis products was carried out using a non-polar gas chromatograph column Rxi5Sil MS (30 m × 0.25 mm × 0.5 µm, Restek), with helium as the carrier gas (1 mL/min) and an oven ramp (initial temperature of 50 °C maintained for 10 min, raised by 2 °C/min to 320 °C, the final temperature, maintained for 13 min). The gas chromatograph was connected to a mass spectrometer (5977B, Agilent), which was used in the scan mode and operated in electron ionization (electron impact source 70 eV; 230 °C). The analysis was performed with a quadrupole mass spectrometer, working at 2 scans/s from 35 to 700 m/*z*.

Compounds were identified on the basis of their mass spectra, retention times, and comparison with the Wiley mass spectra library (v 2.73) and with published mass spectra. Peaks were integrated using Agilent Masshunter (Version B. 09.00) on the total ion current trace and the relative contribution of each pyrolysis product was calculated as the area of the product over the sum of the peak areas of all of the pyrolysis products.

Pyrolysis products were classified into biochemical categories based on previous publications (Barré et al. 2018; Derenne and Quénéa 2015; Dignac et al. 2005; El Hayany et al. 2021; Lejay et al. 2016; Lejay et al. 2019; Vidal et al 2016). Their most probable origin were from (i) higher plants (lignin, long chain fatty acids, long chain alkanes, cutine and/or suberine derived compounds), (ii) microorganisms (short chain fatty acids and short chain alkanes), (iii) multiple sources (nitrogenous compounds, carbohydrates), (iv) an unspecific origin: pyrolysis products derived from aliphatics, aromatics (phenols, benzenes, polyaromatics) and N-heterocylic compounds, i.e. triazine as a probable by-product from TMAH (Templier et al. 2005). Diversity indices of pyrolysis products were estimated using the effective Simpson index of pyrolysis products (Jost 2007; Lagkouvardos et al. 2017).

Table 1 *Salix* varieties planted in the long-term field trial established in 2001 at the site Pustnäs near Uppsala in central Sweden

Name of *Salix* varieties	Clone number	*Salix* varieties	Taxonomic separation at the species level
Björn	SW 910006	*S. schwerinii* E. Wolf x *S. viminalis* L	*S. viminalis*
Tora	SW 910007	*S. schwerinii* x *S. viminalis*	
Tordis	SW 960299	*(S. schwerinii* x *S. viminalis)* x *S. viminalis L*	
Jorr	SW 880013	*S. viminalis*	
Gudrun	SW 940598	*S. burjatica* Nasarow x *S. dasyclados* Wimm	*S. dasyclados*
Loden	SW 890129	*S. dasyclados*	

Statistics

Rstudio (Version 1.3.1073—© 2009–2020 Rstudio, Inc, https://www.r-project.org/) was used for all statistical analyses and plots. The level of significance for the following statistical analyses was set at $P \leq 0.05$. Differences in means of soil N and organic C contents were tested using a one-way analysis of variance with block (the block effect was the "No. of main plots" of the field trial), first among "*Salix* varieties" and then among "*Salix* species". This was followed by a Tukey honest significant difference post hoc test (implemented in the "agricolae" library version 1.3–7, https://CRAN.R-project.org/package=agricolae). The pH values were not normally distributed and therefore differences in medians among "*Salix* varieties" and "No. of main plots" were analysed using a one-way Kruskal–Wallis test followed by a Dunn´s test of multiple comparisons (implemented in the "FSA" library version 0.9.3, https://github.com/fishR-Core-Team/FSA).

The profiles of pyrolysis compound classes and full mid-IR spectral range were analysed using a principal component analysis (PCA) where the data were centered and scaled, followed by between class analysis (BCA) in order to determine whether the *Salix* varieties affected the profiles (implemented in the ade4 library version 1.7–19) (Thioulouse et al. 2018). The relationships between the profiles of pyrolysis compound classes and full mid-IR spectral range were assessed using Mantel tests on the respective distance matrix (implemented in the vegan library version 2.6–2, https://CRAN.R-project.org/package=vegan).

The values of the effective Simpson index of identified pyrolysis products among "*Salix* varieties" had different variances and therefore, differences in means were tested using a Welch's one-way analysis of variance followed by a Games-Howell post hoc test (implemented in the rstatix library version 0.7.0, https://CRAN.R-project.org/package=rstatix) (Games and Howell 1976).

The fertilisation treatments in the field trial were not randomised, as the fertilised and unfertilised main plots were grouped together (Fig. 1a). However, the monoclonal subplots were randomised within each main plot. Therefore, we analysed the effect of *Salix* varieties on soil organic matter composition, soil organic C and N contents and pH in the unfertilised and fertilised treatments separately. The resulting number of replicates for each willow variety was four in both the unfertilised and fertilised treatments.

Results

General soil properties

Soil total organic C content increased by a factor of approximately 1.3 relative to values at the beginning of the field trial in 2001, but total N content remained constant in both the fertilised and unfertilised treatments (Fig. 2 and Table 2). However, among *Salix* varieties, no significant differences in soil organic C or N contents, or pH were observed in both the fertilised and unfertilised treatments (Table 2).

At the species level, a small but significant effect of *Salix* species on soil organic C content was observed in the fertilised treatment ($P = 0.035$), but not in the unfertilised treatment (Table 2). On average *S. dasyclados* varieties had slightly more soil organic C (0.1%) than *S. viminalis* varieties, and no effects on the soil N content or pH were observed (Table 2).

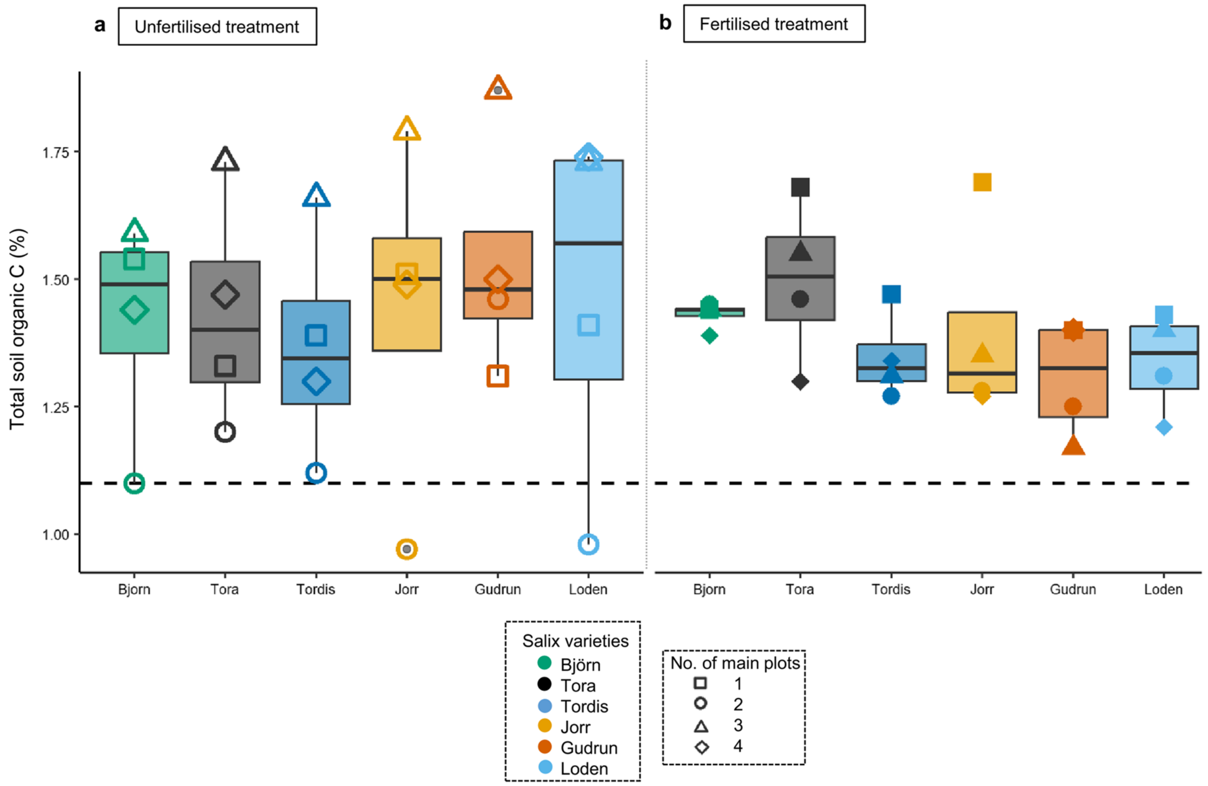


Fig. 2 Total soil organic C (%) in samples from different willow monoclonal subplots: (**a**) the four unfertilised and (**b**) the four fertilised main plots. Boxplots show (i) the median as crossbars inside boxes, (ii) 75th and 25th percentile as crossbars on the top and the bottom of boxes respectively, (iii) the 99th and 1.st percentile as error bars above and below the boxes respectively ($n = 4$). The dashed line indicates total soil organic C (%) which was on average 1.1% ($n = 4$) at the start of the field trial (Weih and Nordh 2005)

Among the main plots there were, however, significant differences in soil total N and organic C contents in the unfertilised treatment ($P < 0.0001$), with less pronounced but significant differences in the fertilised treatment ($P < 0.05$) (Fig. 2 and Table 2). Similarly, soil pH varied significantly among the main plots, but only in the unfertilised treatment ($P < 0.01$) (Table 2). Positive and significant relationships between soil pH, soil N and organic C contents (adjusted $R^2 > 0.5$, $P < 0.0001$) were found in the unfertilised treatment plots, but not in the fertilised treatments.

Composition of soil organic matter below *Salix* varieties: mid-IR spectra

The Between Class Analysis (BCA) of the mid-IR spectra did not reveal any significant separation between *Salix* varieties in the unfertilised treatment (Fig. 3a), but a significant separation was found in the fertilised treatment ($P < 0.01$; Fig. 3b). The variable "*Salix* varieties" explained 22.3% and 24.4% of the overall inertia of the principal component analysis of the unfertilised and fertilised treatments, respectively (Fig. 3).

Overall, in the fertilised treatment, the composition of soil organic matter below Loden and Jorr varieties differed from the other four varieties (Figs. 3b, S1). Loden and, to a lesser extent, Jorr were separated from Björn, Tora, Tordis and Gudrun along the horizontal axis due to more abundant aromatic C-H out-of-plane bending with a high degree of substitution (wavenumbers ranging from 895 to 909 cm^{-1}, 950–970 cm^{-1} and 995–1005 cm^{-1}), C=C aromatic (wavenumbers 1536 cm^{-1} and 1611–1613 cm^{-1}) and aliphatic C-H stretch (wavenumbers 2946 cm^{-1}). Furthermore, soil organic matter below Loden and,

Table 2 Soil characteristics of the long-term field trial

Soil treatments	Factors	Total organic C* content (%)	Total N* content (%)	pH (H_2O)
Unfertilised	*S. viminalis*	1.4^{a}	0.12^{a}	6.7^{a}
	S. dasyclados	1.5^{a}	0.13^{a}	6.7^{a}
Fertilised	*S. viminalis*	1.4^{a}	0.12^{a}	6.0^{a}
	S. dasyclados	1.3^{b}	0.11^{a}	5.9^{a}
Unfertilised	Björn	1.4 ^{a}	0.12 ^{a}	6.4 ^{a}
	Tora	1.4 ^{a}	0.12 ^{a}	6.6 ^{a}
	Tordis	1.4 ^{a}	0.12 ^{a}	6.4 ^{a}
	Jorr	1.4 ^{a}	0.12 ^{a}	6.6 ^{a}
	Gudrun	1.5 ^{a}	0.13 ^{a}	6.5 ^{a}
	Loden	1.5 ^{a}	0.13 ^{a}	6.4 ^{a}
Fertilised	Björn	1.4 ^{a}	0.12 ^{a}	6.0 ^{a}
	Tora	1.5 ^{a}	0.13 ^{a}	6.2 ^{a}
	Tordis	1.4 ^{a}	0.11 ^{a}	6.0 ^{a}
	Jorr	1.4 ^{a}	0.12 ^{a}	5.9 ^{a}
	Gudrun	1.3 ^{a}	0.11 ^{a}	6.0 ^{a}
	Loden	1.3 ^{a}	0.11 ^{a}	5.9 ^{a}
Unfertilised	Main plot 1	1.4 ^{b}	0.12 ^{b}	6.5 ^{ab}
	Main plot 2	1.1 ^{a}	0.10 ^{a}	6.3 ^{a}
	Main plot 3	1.7 ^{c}	0.14 ^{c}	7.8 ^{b}
	Main plot 4	1.5 ^{b}	0.13 ^{cb}	6.5 ^{ab}
Fertilised	Main plot 1	1.5 ^{b}	0.13 ^{b}	5.8 ^{a}
	Main plot 2	1.3 ^{a}	0.11 ^{a}	5.9 ^{a}
	Main plot 3	1.4 ^{ab}	0.11 ^{a}	6.1 ^{a}
	Main plot 4	1.3 ^{a}	0.11 ^{a}	6.1 ^{a}

Means of total N and organic C contents and medians of pH are presented for four field replicates. Means and medians suffixed by a different letter are significantly different at $P < 0.05$

[*] At the start of the experimental field trial in 2001, total organic C and total N contents were on average 1.1% and 0.12% respectively and the bulk density was on average 1.3 g cm^{-1} ($n = 4$) across the trial (Weih and Nordh 2005)

to a lesser extent, Jorr were depleted, relative to the other varieties, in carbohydrate groups C–OH (wavenumbers ranging from 1160 to 1185 cm^{-1}), carboxylic acid C-O stretch, OH deformation, ester, phenol C-O asymmetric stretch (wavenumbers 1201 to 1273 cm^{-1}), ester C-O stretch (wavenumbers 1321 to 1323 cm^{-1}), amide C=O strectch (amide I) (wavenumbers 1652 to 1660 cm^{-1}) and C=O carboxylic (wavenumbers 1644–1646 cm^{-1} and 1718 cm^{-1}).

In the unfertilised treatment, the mid-IR spectra did not differ among the six *Salix* varieties (Fig. 3a), but did so significantly among the four main plots ($P < 0.01$; Fig. S2). Here, the calcite stretches (wavenumbers 2520 to 2543 cm^{-1}), carbonyl C=O stretch (wavenumbers 1705 to 1732 cm^{-1}, 1746 cm^{-1}, 1757–1763 cm^{-1}) and carboxylic acid C-O stretch, OH deformation, ester, phenol C-O asymmetric stretch (wavenumbers 1225 to 1275 cm^{-1}) were most abundant in the third main plot and least abundant in the second main plot (Fig. S2). Changes in mid-IR spectra between the four main plots were similar to changes in other general soil properties such as pH and soil organic C (adjusted $R^2 > 0.5$; $P < 0.0001$).

Composition of soil organic matter below *Salix* varieties: pyrolysis-GC/MS

The BCA of the pyrolysis-GC/MS did not reveal any significant separation *Salix* varieties in the unfertilised treatment (Fig. 4a), but a significant separation in the fertilised treatment was found ($P < 0.03$; Fig. 4b). The variable "*Salix* varieties" explained 16.5% and 34.8% of the overall variation in the unfertilised and fertilised treatments, respectively (Fig. 4). The composition of soil organic matter below Gudrun differed the most from the varieties Tora and Jorr in the fertilised treatment (Figs. 4b, S3 and Table 3).

Gudrun and, to a lesser extent, Loden were separated from the other varieties along the horizontal axis due to a higher abundance of phenol derivatives

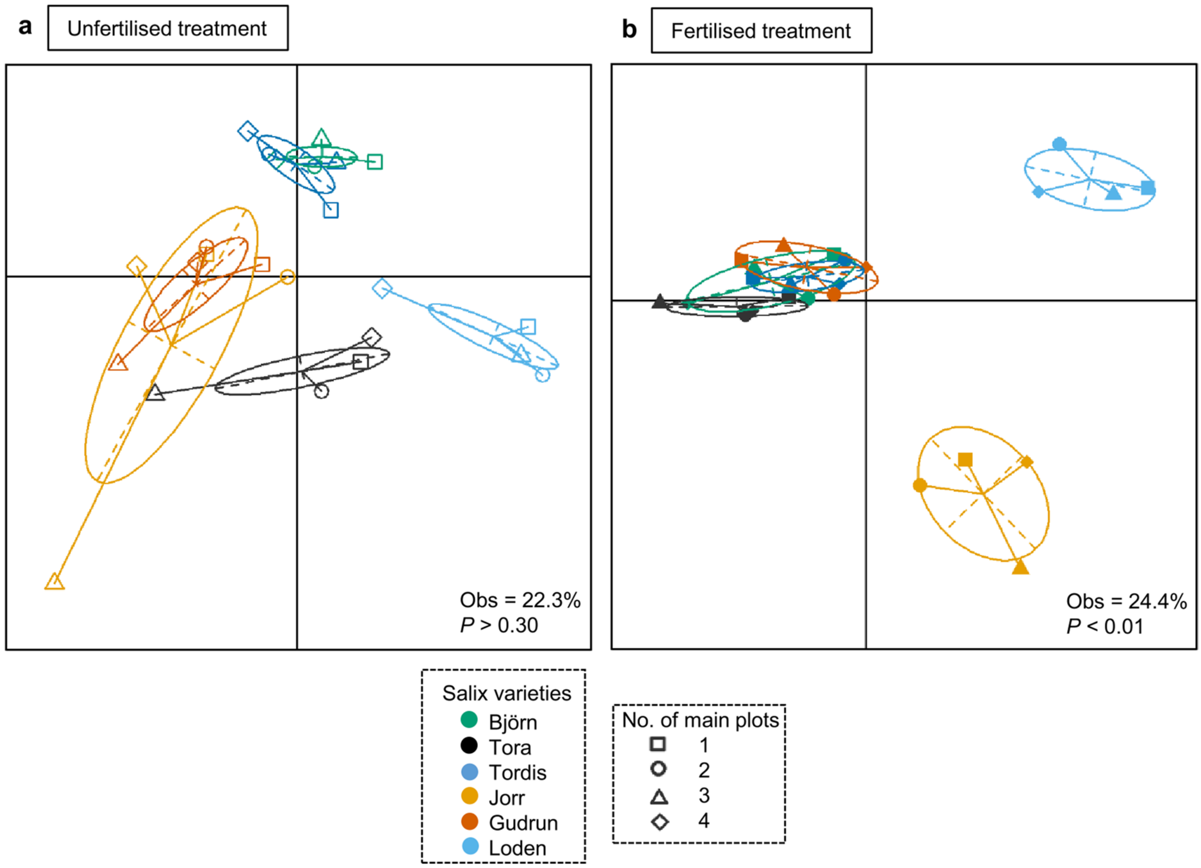


Fig. 3 Between-class analysis (BCA) of the full mid-infrared spectra of the soil from beneath the different *Salix* varieties: (**a**) unfertilised treatment and (**b**) fertilised treatment. The abbreviation "Obs" refers to the percentage of the overall inertia in the data explained by the variable "*Salix* varieties"

of unspecific origin. Furthermore, Gudrun was enriched in nitrogenous compounds from proteins and nucleic acids compared to Tordis and Tora. Carbohydrates and lignin derived compounds from higher plants were enriched in Gudrun compared to Tora. Loden was depleted in short chain fatty acids derived from microorganisms compared to Tora. Loden and Gudrun clearly had a lower abundance of N-heterocylic compounds of unspecific origin compared to Tora, Björn and Tordis.

The separation along the vertical axis between Jorr and the other varieties was due to more aliphatic compounds of unspecific origin and more abundant long chain fatty acids of higher plant origin, particularly in comparison to Gudrun and Loden. Specifically, among the aliphatic compounds of unspecific origin, the most abundant pyrolysis product in soil beneath Jorr was derived from acetic acid. Furthermore, soil organic matter below Jorr was depleted in benzene derivatives of unspecific origin relative to the other varieties.

Although soil organic matter below the six varieties was composed of similar compound classes differing only in relative abundances, we observed that the diversities of identified pyrolysis products were different among varieties in the fertilised treatment: Gudrun had a significantly higher effective Simpson index compared to Tora and Tordis ($P < 0.001$) and Jorr ($P < 0.05$), by a factor of about 1.7 (Fig. 5).

In the unfertilised treatment, we did not observe any differences in pyrolysis-GC/MS profiles or diversity of pyrolysis products between varieties or between the four main plots.

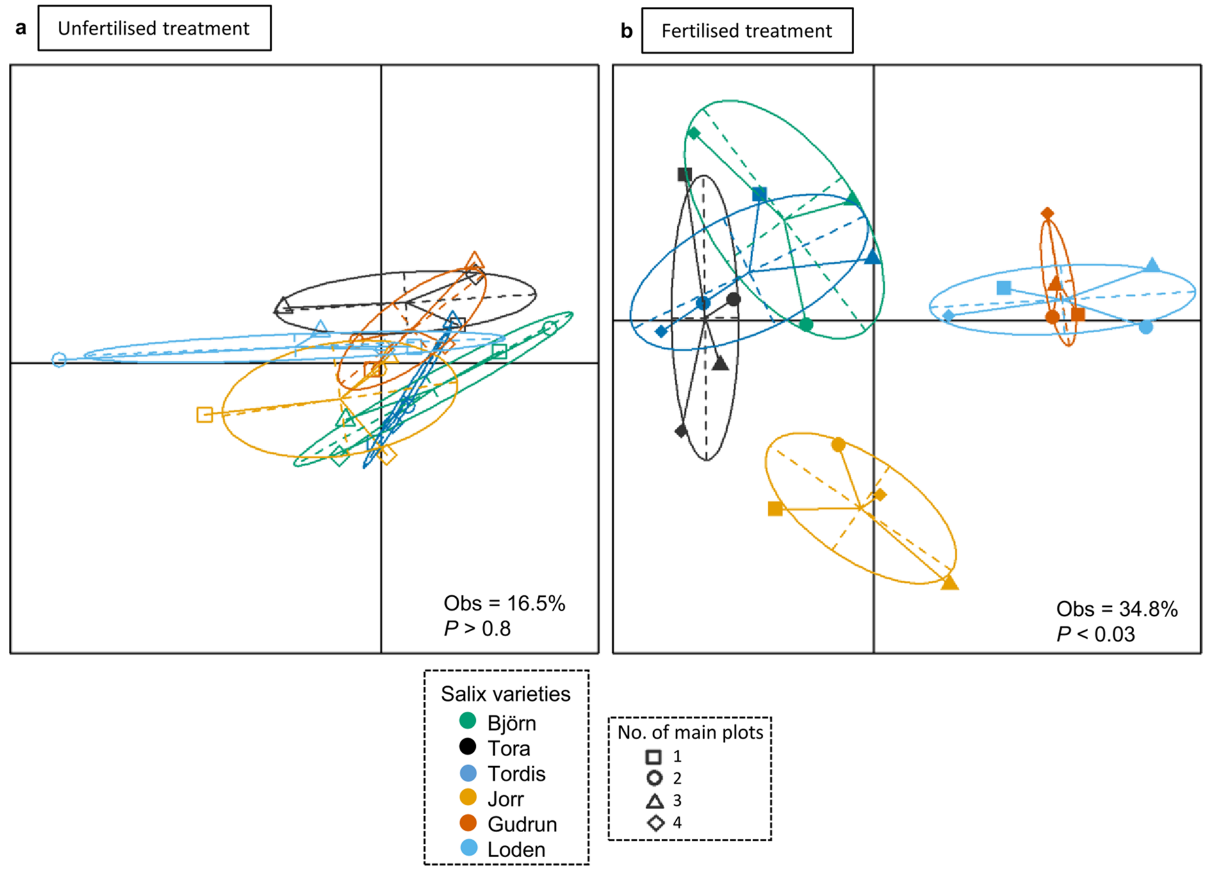


Fig. 4 Between-class analysis (BCA) of identified pyrolysis products of soil organic matter among different willow monoclonal subplots for (**a**) 23 soil samples in the unfertilised treatment (one NA value for Tora main plot 2) and (**b**) 23 soil samples in the fertilised treatment (one NA value for Björn main plot 1). Only the BCA ordination on samples from the fertilised treatment was significant. The abbreviation "Obs" refers to the percentage of the overall inertia in the data explained by the variable "*Salix* varieties"

Table 3 Biochemical categories of identified pyrolysis products from the soils in the fertilised treatment

Most probable origin	Biochemical categories	Björn %	Tora	Tordis	Jorr	Gudrun	Loden
Higher plant	Lignin	11 ± 1	10 ± 2	11 ± 4	13 ± 3	14 ± 1	15 ± 4
	Long chain fatty acids	3 ± 1	2 ± 1	3 ± 1	3 ± 0	2 ± 1	2 ± 1
Microbial community	Short chain fatty acids	4 ± 1	6 ± 1	5 ± 2	5 ± 1	4 ± 1	4 ± 1
Multiple origins	Nitrogenous compounds	6 ± 3	5 ± 2	5 ± 2	6 ± 1	8 ± 2	7 ± 2
	Carbohydrates	6 ± 2	3 ± 1	4 ± 2	4 ± 1	5 ± 1	5 ± 1
	Other aliphatics	12 ± 10	18 ± 7	15 ± 8	30 ± 3	21 ± 3	21 ± 4
	Phenols	1 ± 1	1 ± 1	1 ± 1	1 ± 0	3 ± 0	3 ± 1
	Benzene derivatives	16 ± 11	19 ± 11	20 ± 8	10 ± 1	18 ± 7	20 ± 5
	N-heterocyclic compounds	40 ± 14	34 ± 2	34 ± 8	25 ± 5	22 ± 2	21 ± 1

The relative abundances expressed as % are shown as the mean ± one standard deviation for four replicates (except for Björn that has one NA value). Polyaromatic compounds, cutin and/or suberine originated compounds, short chain alkane and long chain alkane are not presented because their relative abundances were similar among varieties

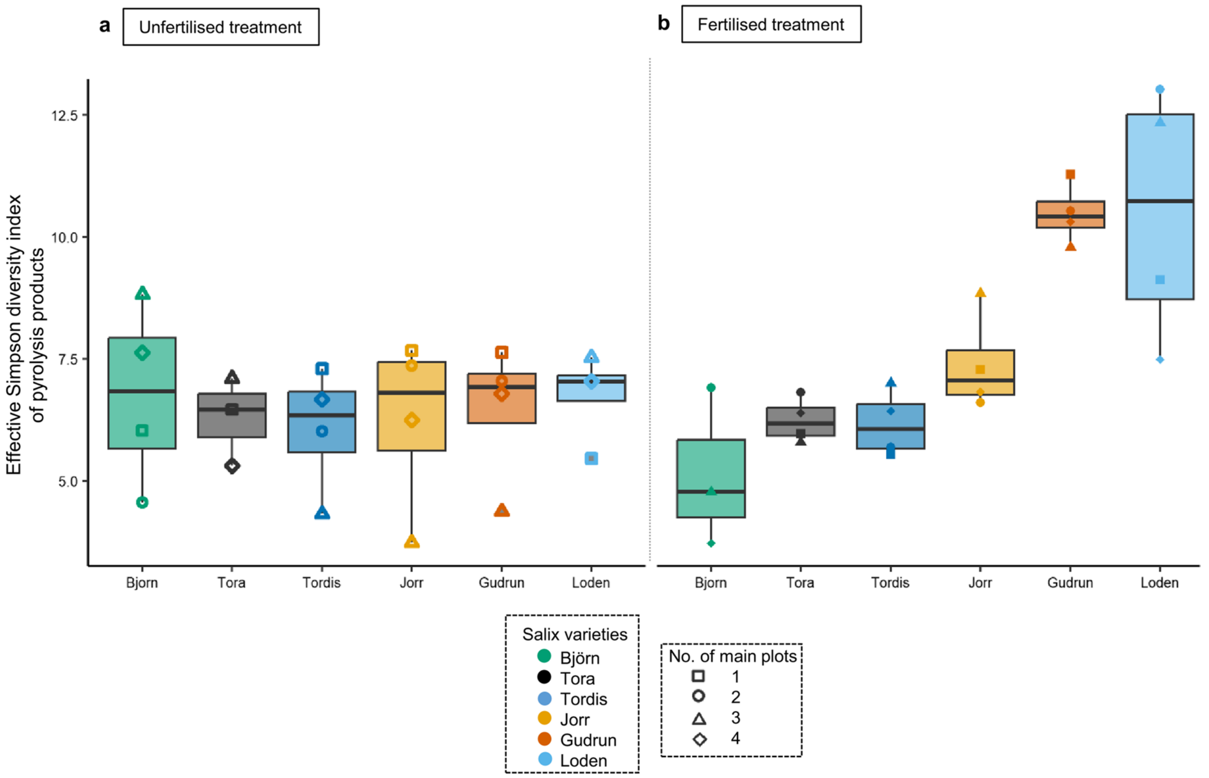


Fig. 5 Effective Simpson diversity index of identified pyrolysis products of soil organic matter from different willow monoclonal subplots: (**a**) unfertilised treatment and (**b**) the fertilised treatment. Boxplots show (i) the median as crossbars inside boxes, (ii) 75th and 25th percentile as crossbars on the top and the bottom of boxes respectively, (iii) the 99th and 1.st percentile as error bars above and below the boxes respectively ($n=4$, except for Tora in the unfertilised main plot 2 and for Björn in the fertilised main plot 1)

Discussion

The link between soil organic matter and inputs from vegetation (rhizodeposition and litter) has long been established for different types of plant cover and plant diversities. For example, diverse plant communities tend to increase soil C stocks relative to monocultures (Chen et al. 2020). However, the links between soil organic matter and individual plant varieties or species are less well studied. It is important to understand the effects that plant varieties or species can have on soil organic matter as it can aid decision making when selecting plants for managing soil organic matter. This study looked at the content and composition of soil organic matter under different *Salix* varieties that were either fertilised or left unfertilised. The unfertilised plots showed a high degree of spatial variability in many soil properties (pH, total N and total organic C contents) (Table 2) which are likely to have masked any potential differences that might have occurred among varieties. As a result, no significant effects of *Salix* varieties or species on the molecular composition and diversity of soil organic matter were observed in the unfertilised treatment. The variability in the unfertilised plots may come from an edge effect due to the proximity of plot 2 to the adjacent wooded area (Fig. 1), and plot 3 had slightly different properties (Table 2 and Fig. S2), all of which increased the interplot variability. The fertilised plots were less variable and significant differences were observed. Therefore, the following discussion only refers to the results obtained in the fertilised plots.

Effects of *Salix* varieties and species on the amount of soil organic matter

Even though there were differences in aboveground traits (biomass, N content and yield) among varieties

(Weih and Nordh 2002, 2005), which might affect the organic matter inputs to the soil (Hirte et al. 2018), no differences in total soil organic C content among varieties were observed (Fig. 2 and Table 2). This is contrary to what was found by Baum et al. (2020). The latter study analysed the surface 10 cm whereas the surface 20 cm were analysed here, which might explain the divergent results. Although it has been observed that about half the root biomass of *Salix* is found in the top 10 cm (Heinsoo et al. 2009), a significant proportion of the rooting system is found at greater depths (Chimento and Amaducci 2015). Therefore, we decided to sample soils down to 20 cm in this study. Nevertheless, the greater sampling depth may have diluted any potential varietal signal. The difference between the results of Baum et al. (2020) and those obtained here suggests that a varietal effect, although weak, might be greater in the upper 10 cm of the soil. This is in line with the work of Martani et al. (2021) where the authors observed a positive rate of soil organic C sequestration in the 0–10 cm layer for willow but either no effect or a negative effect in the 10–30 cm soil layer.

At the species level, *S. dasyclados* varieties had significantly lower total soil organic C content than *S. viminalis* varieties (Table 2). This suggests that the differences in traits among species rather than varieties were sufficiently large to affect the amount of soil organic C. The accumulated shoot C in the fertilised plots of the four *S. viminalis* varieties was approximately twice that of the two *S. dasyclados* varieties (Rönnberg-Wästljung et al. 2022). *S. dasyclados* varieties tend to have both higher fine root biomass (Heinsoo et al. 2009) and higher ectomycorrhizal colonisation than *S. viminalis* (Püttsepp et al. 2004). On the one hand, roots that are colonised by ectomycorrhiza tend to be decomposed less rapidly than roots that are not mycorrhizal (Langley et al. 2006). On the other hand, the abundance of some genera of ectomycorrhizal fungi (i.e. *Russela* and *Cortinarius*), that are capable of producing extracellular peroxidase, have been shown to correlate negatively with the proportion of soil organic matter associated with minerals (Hicks Pries et al. 2023). *S. dasyclados* varieties are particularly colonised by *Cortinarius* spp., a morphotype that has been associated with reduced soil organic matter contents in boreal forests (Lindahl et al. 2021), potentially via the production of manganese-peroxidases (Kellner et al. 2014). No such colonisation of *S. viminalis* has been found (Püttsepp et al. 2004). However, it should be noted that *Cortinarius* spp. abundances (Jörgensen et al. 2022) and peroxidase activity (Bödeker et al. 2014) can be reduced by N fertilisation and therefore this interpretation might not be pertinent for forest soils. Yet, here in the context of arable land, this explanation may still remain relevant (BD Lindahl, personal communication).

Effects of *Salix* varieties and species on composition of soil organic matter

The most significant result obtained in this study is that the taxonomic proximity of the *Salix* varieties affected the molecular diversity and composition of the soil organic matter, as seen in the pyrolysis-GC analyses (Figs. 5, S3). Even though there is no simple way of quantifying the taxonomic distance of the *Salix* varieties (Fogelqvist et al. 2015), Loden and Gudrun are separated taxonomically at the species level from all other varieties (Table 1; Weih and Nordh 2005). Loden is a pure *S. dasyclados* clone whereas Gudrun contains two species, namely *S. burjatica* and *S. dasyclados*. Jorr is a pure *S. viminalis* clone, Tordis is derived from two species (*S. schwerinii* and *S. viminalis*) and Björn and Tora are full-siblings. The latter two are therefore expected to behave in very similar way in an ecological context.

Hypothetically, the taxonomic proximity of the varieties may reflect a proximity of traits. Previous studies have suggested that *S. viminalis* varieties differ from *S. dasyclados* varieties in the following characteristics: (i) higher aboveground biomass yields (Kalita et al. 2021), (ii) higher sodium concentrations in leaves (Ågren and Weih 2012), (iii) higher contents in catechin and rutin (quercetin 3-*O*-rutinoside), lower naringenin and salicylic acid concentrations (Curtasu and Nørskov 2024), (iv) lower lignin contents (Kalita et al. 2023), (v) lower leaf area ratios, lower leaf area productivity (Weih and Nordh 2002), (vi) lower leaf N content (Hoeber et al. 2017), (vii) lower ectomycorrhyzal but higher arbuscular mycorrhizal colonization (Püttsepp et al. 2004), (viii) lower fine root biomass (Baum et al. 2018). Yet, Hoeber et al. (2020) showed that the variability of leaf litter decomposition across the four *S. viminalis* (Björn, Tora, Tordis and Jorr) did not strictly follow the taxonomic proximity hypothesis in relation to remaining mass and N. Due to the complexity of the relationship between above- and belowground inputs and soil organic matter properties (Kögel-Knabner 2017), it is not possible to say which, if any, of these traits are responsible for the differences in composition of soil organic matter that were found here. Although it is likely to be a combination of a number of them.

Most of these traits are not clearly reflected in the pyrolysis product profiles of soil organic matter. However, the soil organic matter under the *S. dasyclados* varieties contained more lignin compared to *S. viminalis*, possibly due to the higher lignin content of their aboveground biomass and the relatively lower decomposition rates of lignin compared to other constituents of the plant litter (Hall et al. 2020a). The higher phenolic compound concentrations under *S. dasyclados* are likely related to the lignin contents, as phenolic compounds are formed upon the pyrolysis of lignin (Dignac et al. 2009).

The differences in molecular diversity may be due to a combination of greater organic C inputs from *S. viminalis* varieties and greater microbial processing of the organic matter inputs in soil under *S. dasyclados* varieties. Others have found that microbial and enzymatic processing of organic matter can dramatically increase its molecular diversity (Kallenbach et al. 2016; Wang et al. 2023). In addition, a negative relationship between root biomass and the molecular diversity of soil organic matter has been found suggesting that higher inputs decrease molecular diversity (Wang et al. 2023).

Even though most of the differences in molecular composition and diversity of the soil organic matter were seen between varieties of different species, the soil organic C under Jorr also differed from that in the other *S. viminalis* varieties. Jorr biomass contains more cellobiose, galactose and arabinose, but contained less xylose and had a lower biomethane potential than other *S. viminalis* varieties (Kalita et al. 2023). The major differences in acetic acid derived compounds under Jorr may be due to differences in the composition of the hemicellulose monomer profiles in its above-ground biomass (Kalita et al. 2023). Acetic acid derived compounds can have multiple origins but the cleavage of hemicellulose acetyl groups is among them (Pouwels et al. 1987). The aliphatic region of the IR spectra and the aliphatic contents (long chain fatty acids and other aliphatics) obtained by pyrolysis tended to be slightly higher in Jorr than in the other varieties (Figs. S1, S3 and Table 3). It has been suggested that these may be indicators of plant derived organic matter with a molecular structure dense in C-H bonds such as in waxes from leaf litter or some root exudates dense in hydrocarbon bonds (Mainka et al. 2022).

To our knowledge, this is the only study assessing soil organic matter composition and diversity under willow plantations after as much as 18 years. Nevertheless, 18 years is relatively short compared to the mean turnover times of top soil organic C, which is in the order of decades (Sierra et al. 2018). Whilst the age of this long-term field experiment does not allow us to extrapolate changes in composition over the longer term, the changes in the composition of the organic matter may be seen as indicative for the changes over the longer term.

Comparison between pyrolysis GC/MS and mid-IR analyses

Compared to the pyrolysis GC/MS method, the mid-IR spectral analysis approach is simpler, cheaper and has the advantage of being non-destructive. We were therefore interested in determining whether mid-IR spectral analyses could be used to determine differences in the composition of the organic matter in soil under different varieties of *Salix*. Although the mid-IR method discriminated certain varieties from others (Figs. 3, S1), the discrimination was not identical to that found with pyrolysis. Indeed, a Mantel test showed that the two methods were not closely related (data not shown). The divergence between the two methods may be due to the fact that the mid-IR analysis discriminated Jorr and Loden from the other varieties while the pyrolysis analysis mainly discriminated Gudrun and Loden from the other varieties.

Nevertheless, there were some similarities: for example, both the mid-IR and pyrolysis data suggest that the carbohydrate content of soil organic matter beneath Jorr was lower than that in Gudrun and, as indicated above, similarities were also seen in the aliphatics/long chain fatty acids. These similarities may be due to the relatively high variation of these molecular groups within our data and between the varieties (Table 3 and Figs. S1, S3), allowing the mid-IR spectral analysis to detect them.

Conclusions

This study provides evidence that the identity of *Salix* species or varieties can affect the molecular composition and diversity of soil organic matter. The corresponding traits should be considered in breeding programmes of biomass willows to enhance the organic C accumulation and persistence in the soils that these willows are grown on. It would be interesting to determine whether these results are maintained or amplified in diversified systems, i.e. where different varieties are grown together.

Acknowledgements This research was funded by the Swedish Research Council for Sustainable Development, FORMAS projects (22836000, MixForChange 2020-02339 and OPTUS 22551000). The authors would like to thank N.-E. Nordh for assistance with the field sampling, C. Baum, P. Barré, P. Smith and S. Manzoni for discussions related to the long-term field trial, material preparation, data collection and analysis to carry on, M. Spångberg, E. Ljunggren for assistance with laboratory work, E. Karltun and D. Wasner for assistance with mid-IR analysis as well as J. Forkman for assistance with statistical analysis. Thanks to E. Pihlap, M. Ota and Y. Tian for valuable comments on the manuscript. We also thank two anonymous reviewers for their pertinent and constructive comments, which contributed to the improvement of the manuscript.

Author contributions A.M.H. and M.W. conceptualized the study. L.J.P.D. and A.S. collected the data. L.J.P.D. and N.N. performed statistical analyses. J.W. gave advices on how to analyse mid-IR data. K.Q. performed the pyrolysis GC/MS analysis. All authors contributed to manuscript development and revisions.

Funding Open access funding provided by Swedish University of Agricultural Sciences. This work was supported by the Swedish Research Council for Sustainable Development, FORMAS project (22836000, MixForChange 2020-02339 and OPTUS 22551000).

Data availability The datasets generated during the current study and the custom R scripts used for data analysis are available from the corresponding author on reasonable request and from the public repository entitled Zenodo (https://zenodo.org/records/10906904) (Creative Commons Attribution 4.0 International).

Declarations

Competing interests The authors declare they have no relevant financial or non-financial interests to disclose.

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