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# Carbon dynamics in soils – Does the diversity of organic inputs matter?

## A bioenergetics approach

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Cover: A hotspot of soil activity that harbours a community of organisms thriving on mineral and organic surfaces. A digital image was first generated by DALL-E, a text-to-image model, using the above keywords as an artistic representation of an image taken by an electronic microscope, and then converted into a black and white drawing using the 'draw by number' method. The final drawing was created using colour pencils by Oksana Valetska.

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## Abstract

The diversification of agroecosystems is an attractive agricultural practice that can lead to climate change mitigation/adaptation by stimulating carbon sequestration. As the chemical composition of plants can vary, the diversification of plant communities can impact the chemical properties of the organic inputs to soil. The changes in the chemical properties of organic inputs to soil, such as their molecular diversity, may influence both microbial activity and the composition of soil organic matter. Therefore, the overall aim of this thesis was to investigate how diversified organic inputs affect the soil microbial processing. Specifically, how do diversified organic inputs to soils affect below-ground organic matter properties and how does this affect soil microbial activity? Topsoil samples from various soil systems from cereal dominated agriculture to woodland, including a long-term field trial with *Salix*, were used in the different studies. The chemical composition of soil organic matter was characterised, and the energy content of hot water-extractable organic matter and its nominal oxidation state were determined. Microbial activity was analysed by measuring heat dissipation from soil by isothermal calorimetry, and microbial energetic return on investment was calculated. The activity of soil microbial communities is related to the properties of the available soil organic matter, most notably the potential return on energetic investment that the microbial communities can obtain when consuming the organic substrates. The diversity of the available substrates did not seem to affect microbial activity, but their average energetic properties did. Microbial activity reached a maximum when microorganisms processed substrate mixtures with redox properties that matched their preferences. Only at low levels of molecular diversity, microbial community happened to process substrate mixtures in a non-additive, synergistic manner. The longer-term fate of the C inputs may also be affected by the characteristics of the organic inputs: the composition and diversity of soil organic matter was influenced by *Salix* species, but not by varieties, when not confounded by the heterogeneity in soil properties. Soil C dynamics can only be fully understood through the prism of interactions between organic substances and microorganisms and may not be modulated by managing the diversity of organic inputs but rather by influencing their intrinsic energetic properties.

*Keywords:* Diversification, community composition, soil, organic matter, microbial activity, return on investment, bomb & isothermal calorimetry, spectroscopy, mass spectrometry

# Koldynamiken i marken – Spelar variationen av det organiska materialet någon roll?

## Sammanfattning

Diversifiering av agroekosystem är en attraktiv jordbruksmetod som kan leda till begränsning/anpassning av klimatförändringar genom att stimulera kolinlagring. Variation i kemiska materialegenskaper som tillförsel marken kan påverka både mikrobiell aktivitet och sammansättningen av markens organiska material. Det övergripande syftet med avhandling var därför att undersöka hur variationen i det organiska material som tillförs marken påverkar markens mikrobiella bearbetning av det organiska materialet. Specifikt studerades hur variationen i det organiska materialet som tillförs marken påverkar egenskaperna av markens organiska material, och hur det påverkar den mikrobiella aktiviteten? I studierna användes jordprover från spannmålsdominerad jordbruksmark till skogsmark, inklusive ett långliggande fältförsök med *Salix*. Den kemiska sammansättningen av markens organiska material karakteriserades och det nominella oxidationstillstånd bestämdes i varmvatten-extraherat organiskt material. Mikrobiell aktivitet analyserades genom att mäta värmeutvecklingen från jorden med isotermisk kalorimetri, och mikrobernas så kallade ’energetiska avkastning på investeringen’ beräknades. Aktiviteten hos de mikrobiella samhällena i marken påverkas av egenskaperna hos det tillgängliga organiska materialet i marken, framförallt den potentiella avkastningen på investerad energi som mikrobsanhållet kan få ut genom nedbrytningen av det organiska substratet. Variationen av substrat verkade inte påverka aktiviteten men det gjorde dess energi-egenskaperna. Den mikrobiella aktiviteten nådde ett maximum när mikroorganismerna bearbetade substrat-blandningar med redoxegenskaper som matchade deras preferenser. Endast vid låga nivåer av molekylär diversitet skedde deras nedbrytning synergistisk, dvs. på ett icke-additivt sätt. Den mer långsiktiga effekterna av C-tillförseln kan också påverkas av egenskaperna hos det organiska materialet som tillförs marken: sammansättningen och variationen av markens organiska material påverkades av *Salix*-arter, men inte av sorter, när de inte överskuggades av heterogeniteten i andra markens egenskaper. Koldynamiken i marken kan bara förstås till fullo genom prismat av interaktioner mellan organiska ämnen och mikroorganismer, och kan inte påverkas enbart genom att tillföra ett diversifierad material, utan snarare genom att påverka deras inneboende energetiska egenskaper.

*Sökord:* Mångfald, samhällets sammansättning, jord, organiskt material, mikrobiell aktivitet, energetiskt ’return on investment’, kalorimtetri, spektroskopi, masspektrometri

# Preface

When I was a kid, my grandparents were often telling me stories about when they were younger during the war in Europe: the need to walk a long time to get water to the well, the Jerusalem artichokes, the bombs, the migration, the bell tower of the church, the different colours in the cropland in Beauce where different varieties of a given cereal were sown together. Later, dreams of Rocamadour, Saint Nectaire, Cantal and Roblochon when climbing along steep slopes covered by very porous dark scorias in the Natural Park of the Auvergne or listening to the melody of the cowbell in the Aravis mountain range make me wonder about volcanic or grasslands soils harbouring a wide variety of flowers. At the age of ten, I once got lost in Luxor, absorbed into my fresh memories about the archaeological treasures of the Valley of the Artisans and how some dark yellowish brown stony sandy soils could neighbour more fertile ones along the Nile River. In Corrèze, I often walked with my dogs in the woods, looking for giroles and black trumpets hidden below decomposing chestnut tree leaves on the top of a thin brown reddish organic horizon. In Marrakesh, I was charmed by its bazaar with colourful species, the call of the mosque’s minaret and its Palmeraie harbouring relaxing dromedary on some oasis soils. In Bordeaux, after practising sax scales, faced the synagogue and waiting for my inner seeds to germinate in the soil of a collective urban garden, I was listening to my dear singing *Someone Like You*, *Caruso*, *L-O-V-E*, *Bésame Mucho*, *I Look to You*. In Asia, I was surprised to discover, between the visits to Taoist, Confucianist and Buddhist temples, how red soils could be and how urbanisation could impact these soils. In Strasbourg, crossing the border to Germany without customs to dance tango, salsa or rock’n’roll or biking for home delivery of meals, my professor Benoît Kammerer taught me that microorganisms’ homeostasis, surface area-to-volume ratio, crowded cytoplasm and the miss-match between in vitro and *in vivo* kinetics parameters of enzymes were major aspects often overlooked in microbiology and even more in soil sciences. Since then, loves, losses, tears, fears, joy, hopes, and dreams embellish this journey on the path to diversification of the plant-soil-microbial systems.

# Dedication

*To my brother who escaped death and managed to defend his PhD*

*To my mum who recently felt sorrow and grief*

*To my loved ones who have passed away*

*To my daughter, my son and their mum for their sparkling dreams*

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Dufour, L.J.P.**, Herrmann, A.M., Leloup, J., Przybylski, C., Foti, L., Abbadie, L., Nunan, N. (2022). Potential energetic return on investment positively correlated with overall soil microbial activity. *Soil Biology and Biochemistry*, 173, 108800. https://doi.org/10.1016/j.soilbio.2022.108800

II. **Dufour, L.J.P.**, Nunan, N., Herrmann, A.M. Does substrate diversity affect microbial heat dissipation in soil? (manuscript)

III. **Dufour, L.J.P.**, Shi, A., Wetterlind, J., Nunan, N., Weih, M., Quenea, K., Herrmann, A. (2025). *Salix* species and varieties affect the molecular composition and diversity of soil organic matter. *Plant Soil*. 508, 767-784 https://doi.org/10.1007/s11104-024-06829-x

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The contribution of Louis J.P. Dufour to the papers included in this thesis was as follows:

I. Performed collection of soil sample and laboratory analyses (except for ultrahigh-resolution mass spectrometry measurements of soil organic matter and sequencing of soil bacterial communities). Carried out code writing (except for the TRFu algorithms) and data analysis. Prepared the first draft of the manuscript and wrote the paper with support of the co-authors. Responsible for correspondence with the journal.

II. Planned the study together with the co-authors. Performed laboratory work using isothermal calorimetry and data analysis. Prepared the first draft of the manuscript and wrote the paper with support of the co-authors.

III. Planned the study together with the co-authors. Performed soil sample collection. Performed laboratory work (except for pyrolysis GC/MS measurements). Analysed the data with inputs from co-authors. Prepared a first draft of the manuscript and wrote the paper with support of the co-authors. Responsible for correspondence with the journal.

# Abbreviations

| | |
|---|---|
| C | Carbon |
| N | Nitrogen |
| $CO_2$ | Carbone dioxide |
| SOM | Soil organic matter |
| SOC | Soil organic C |
| NOSC | Nominal oxidation state of carbon |
| WEOM | Water-extractable organic matter |
| Mid-IR | Mid-infrared |
| GC/MS | Gas chromatography – mass spectrometry |
| FT-ICR MS | Fourier-transform ion cyclotron resonance mass spectrometer |
| $\Delta G^0_{Cox}$ | standard molal Gibbs energies of oxidation half reaction [kJ per g C] in Kappa ; [J per mmol C] in Paper I |
| $\Delta E$ | Combustion enthalpy or specific energy content [J $mmol^{-1}$ C] in Paper I ; $\Delta_c H$ [kJ per g C] in Kappa and Paper II |
| ROI | Return on investment |

# 1. Introduction

Soils deliver ecosystem services to human societies (Amundson et al., 2015). These services can contribute to sustainable development goals (Kopittke et al., 2022), such as supporting biodiversity, nutrient cycling, filtering water, producing biomass, and mitigating climate change (Lilburne et al., 2020). Carbon (C) sequestration in soils, which results in a net transfer of C from the atmosphere to soil (Don et al., 2024), is one of the ecosystem services which, if enhanced, might help to mitigate climate change (Roe et al., 2019; Soussana et al., 2019; Baveye and White, 2020). The diversification of agroecosystems, i.e. an increase in the temporal or spatial diversity of the plant community, has been proposed as a potentially attractive agricultural practice for enhancing soil organic C (SOC) storage and is promoted by the EU common agricultural policy (EU CAP). However, it is unclear whether a more diversified agroecosystem leads to a greater potential of SOC sequestration potential.

The modelling of organic matter dynamics in soils (Woolf and Lehmann, 2019; Abramoff et al., 2022; Jarvis et al., 2024) is often used to evaluate the C sequestration potential of soil. However, reliable model predictions of potential C sequestration in soils require the inclusion of model parameters that are based on a clear understanding of the mechanisms involved and related to physicochemical and microbial dynamics (Steffens et al., 2017; Sokol et al., 2019; Li et al., 2024). Empirical studies are therefore needed to constrain models with more detailed data that match model variables (Manzoni and Schimel, 2024).

Soil microorganisms are key players in governing the terrestrial C cycle. In the last decade, microbial bioenergetics has emerged as a new approach in exploring microbial C turnover in soils (Herrmann et al., 2014; Kästner et al., 2024). C processing by microbial communities depends on the type of microbial metabolism and its energetic requirements. The nominal oxidation state of C has been proposed as a universal metric of organic matter to characterise the bioenergetics potential for microbial metabolism (LaRowe and Van Cappellen, 2011; Nunan et al., 2015). As the nominal oxidation state of C relates to both the energy content of organic compounds (Kharasch and Sher, 1925; Kharasch, 1929) and its activation energy in a cellular context (Jinich et al., 2018), it may be used to assess the potential energetic return on investment associated with the metabolic activity of soil microbial communities. The idea that 'return on investment' plays an important role in C dynamics in soils has been suggested previously (Schimel and Weintraub, 2003; Fontaine and Barot, 2005; Williams and Plante, 2018; Henneron et al., 2022) but it is still underutilised in soil science, and empirical data are lacking. The focus of this thesis was therefore to evaluate the impact of diversified organic inputs on microbial C turnover using a bioenergetics approach (Fig. 1).

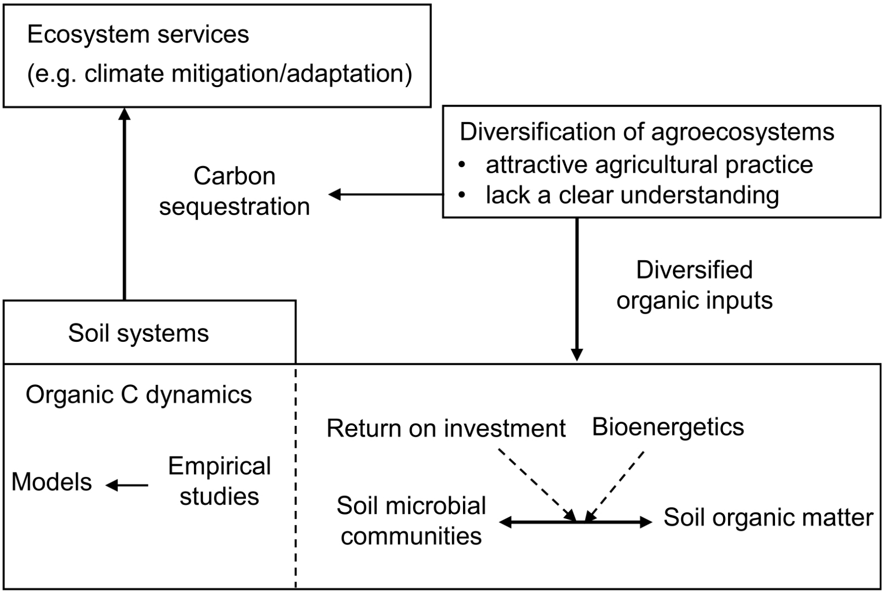


**Figure 1**. Schematic representation of the main problems from which the work of the present thesis is derived.

# 2. Aim, scope and objectives

The overall aim of this thesis was to elucidate how diversified organic inputs affect microbial turnover of organic matter in soils. Specifically, I focused on the transformation of (i) water-extractable organic matter (WEOM, **Paper I**) that should be readily available to soil microorganisms, and (ii) distinct low molecular weight organic compounds with known chemistry, added to soil as a diversity gradient of substrate mixtures (**Paper II**) (Fig. 2). In **Paper III**, the composition of soil organic matter in a long-term field experiment where six *Salix* varieties were grown as short-rotation coppice was evaluated, including a comparison of two routinely used methods.

Soil microorganisms require energy for their maintenance and survival which they can obtain for some of them through the transformation of organic matter. Microbial activity produce heat proportionally to the rate at which chemical reactions or physical processes take place therein. Furthermore, the potential energetic return on investment (ROI) that the microbial community might get during decomposition of soil organic matter are related to abiotic conditions in the soil (Keiluweit et al., 2017; Boye et al., 2018; Bajracharya et al., 2022). The scope of my work was therefore focused on the investigation of the microbial processing of organic material in well-aerated mineral topsoils in temperate climates. Microbial activity was determined using heat dissipation from soil samples and for the quantification of the energetics of microbial reactions in soils (**Paper I** and **Paper II**).

My thesis was based on the following three research questions:

1.) How do diversified organic inputs to soils affect below-ground organic matter properties? (**Papers I** and **III**);
2.) How do diversified organic inputs to soils affect soil microbial activity? (**Papers I** and **II**);
3.) How does the potential energetic ROI that the microbial community can get from processing organic matter affect their activity? (**Paper I**)

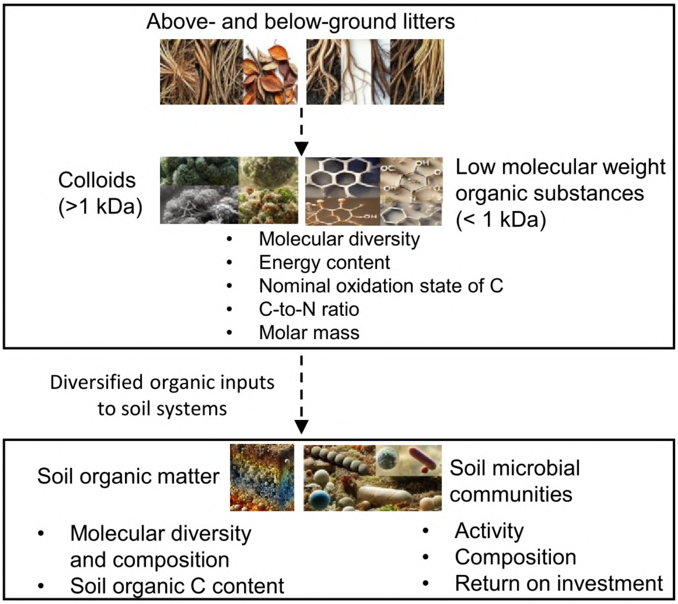


**Figure 2**. Schematic of the scope of this thesis. Digital images have been generated from DALL-E, a text-to-image model

The specific objectives associated with these three research questions were:

- to explore the relationship between the potential energetic ROI and the microbial metabolic activity in response to added organic matter by developing a method suitable for soil science;
- to evaluate potential effect of interactions when soil microorganisms are exposed to a gradient of organic matter varying in heterogeneity;
- to determine whether the molecular composition and diversity of soil organic matter was related to above-ground plant varieties;
- to compare the pyrolysis-gas chromatography mass spectrometry and mid-infrared spectroscopy methods.

# 3. Background

Globally, soils contain more than twice the amount of C present in the atmosphere and three times the amount of organic matter in aboveground biomass. They are therefore major components in the global C cycle. Sequestering atmospheric $CO_2$ into soil organic matter through changes in agricultural practices is an appealing idea that requires less investment than other proposed methods of C sequestration (Hoffert et al., 2002; Kell, 2012; Kin et al., 2019; Hoffland et al., 2020). The chemical composition of organic matter in soils varies widely (Vidal et al., 2021; Meklesh et al., 2022; Delahaie et al., 2024), with the dominant drivers of its composition being similarly variable (Table 1), making predictions in changes of soil organic matter difficult.

**Table 1.** Dominant drivers of chemical composition of soil organic matter.

| **Factors** | **References** |
|---|---|
| Soil type | Kögel-Knabner and Amelung (2021) |
| Climate, soil texture, soil mineralogy | Ding et al. (2020); Hall et al. (2020); Sheng et al. (2023) |
| Soil depth | Soucémarianadin et al. (2018); Roth et al. (2019) |
| Time | Wickings et al. (2012); Mainka et al. (2022) |
| Microbial community | Domeignoz-Horta et al. (2021); Joly et al. (2023) |
| Vegetation type | Wiesmeier et al. (2014); Augusto et al. (2015); Hall et al. (2020) |
| Amount and quality of organic inputs | Pisani et al. (2016); Kögel-Knabner (2017) |

Crop diversification may be an alternative and attractive agricultural practice for enhancing soil C sequestration, while maintaining or increasing crop yields (Beillouin et al., 2021). However, the underlying mechanisms through which plant diversity can increase C storage are not clearly understood. Recently, Lehmann et al. (2020) proposed a theoretical framework in which the persistence of C in soil can be understood as the outcome of interactions between (i) the chemical variability of organic matter and (ii) the '*energy return on investment*' associated with the activity of the soil microbial communities. However, this framework has yet to be tested rigorously. In the following, I would like to scrutinise these two aspects. In addition, this thesis assessed C dynamics in soils using a bioenergetics approach and '*energy return on investment*' requires basic knowledge of bioenergetics. I will therefore start with a brief overview of this area of research.

## 3.1 Bioenergetics in terrestrial ecosystems

Organic matter supplies C, energy and nutrients which are essential for microbial processes to take place in soils. During microbial decomposition, soil organic matter is the electron donor, i.e. providing electrons for catabolic and anabolic microbial processes, in a range of redox reactions. The standard Gibbs free energy of the oxidation half-reaction ($\Delta G^0_{\mathrm{Cox}}$) refers to the oxidation half-reaction of organic matter undergoing decomposition under standard conditions (1 atm, 1M concentration, and 25 °C). However, $\Delta G^0_{\mathrm{Cox}}$ is only one part of redox reactions (Amend and LaRowe, 2019). In soils, $\Delta G^0_{\mathrm{Cox}}$ depends on the composition of organic matter and reduction half reactions are dependent on the soil environment (Table 2). In soils under aerobic conditions, oxygen is often the electron acceptor, while in anaerobic systems less favourable electron acceptors (e.g. Fe(III), $NO_3^{-1}$, $SO_4^{2-}$ etc.) are involved in microbial decomposition of soil organic matter (Table 2) (Zheng et al., 2024). Therefore, to estimate the spontaneity of the overall reaction of decomposition, and the potential energy that microorganisms can harvest, the soil environment must be considered, including the molecular structure and activities of the electron donors and acceptors (Noor et al., 2012; Amend and LaRowe, 2019; West et al., 2024).

The reduction potential describes the tendency of functional groups in molecules to accept electrons. As presented in Table 2, the following functional groups are increasingly oxidised and have an increasing tendency to release electrons: hydrocarbons < hydroxycarbon < carbonyls < and carboxyls. More oxidised C (i.e. higher NOSC) will tend to give electrons more readily and will be characterised by lower reduction potentials (Bar-

Even et al., 2012b; Jinich et al., 2018) and lower $\Delta G^0_{Cox}$. For example, molecules enriched in hydrocarbon functional groups would tend to have a lower NOSC values, higher $\Delta G^0_{Cox}$ values and higher activation energies in a cellular context associated with the oxidation half-reaction of the organic compounds than molecules enriched in carboxylic acid functional groups.

**Table 2.** Redox ladder of key redox reactions in microbial metabolism

| | **Redox half-reaction** | **C oxidation state** | **Reduction potential (mV)** |
|---|---|---|---|
| | $\frac{1}{2}$ $O_2 + 2e^- \leftrightarrow H_2O$ | | ≈ 820 ^ |
| | $Fe(III) + e^- \leftrightarrow Fe(II)$ | | ≈ 760 ^ |
| | $NO_3^- + 2e^- \leftrightarrow NO_2^-$ | | ≈ 420 ^ |
| | $SO_4^{2-} + 8e^- \leftrightarrow H_2S$ | | ≈ -220 ^ |
| | hydroxycarbon + $2e^-$ ↔ hydrocarbon | -2 to +1 ↔ -1 to -4 | ≈ -100 to 50 ! |
| | $CH_2O + 2e^- \leftrightarrow CH_4$ | | ≈ -200 ^ |
| | carbonyl + $2e^-$ ↔ hydroxycarbon or amine | +1 to +2 ↔ -2 to +1 | ≈ -400 to -100 ! |
| | activated carboxyl + $2e^-$ ↔ carbonyl | +3 to +4 ↔ +1 to +2 | ≈ -350 to -250 ! |
| ATP hydrolysis | *Example of spontaneous electron flow* | | |
| | $NAD(P)^+ + 2e^- \leftrightarrow NAD(P)H$ | | ≈ -380 to -250 ! |
| | $CO_2 + 2e^- \leftrightarrow CO$ | | ≈ -596 ! |
| | un-activated carboxyl + $2e^-$ ↔ carbonyl | +3 to +4 ↔ +1 to +2 | ≈ -600 to -500 ! |

! Range of reduction of potentials in physiological conditions ($6 \leq pH \leq 8$ and $0 \leq$ ionic strength $\leq 0.25$ M) (Yishai et al., 2016; Bar-Even et al., 2012b; Jinich et al., 2018). ^ Standard reduction potential (Reeburgh, 1983; Malyan et al., 2016; Plante et al., 2024; West et al., 2024).

In the past five years, theoretical bioenergetics frameworks have been proposed to better understand the dynamics of C, nutrient and energy in soil systems (Chakrawal et al., 2020; Calabrese et al., 2021; Yang et al., 2021; Kästner et al., 2024). These frameworks acknowledge that soil organic matter is the electron donor in microbial decomposition processes in soils, and that the environmental conditions are setting the boundaries for these decomposition processes. However, these frameworks require extensive information from empirical studies when used in soil systems. In the future, there is a clear need to support modelling frameworks with results from empirical studies. For example, three possible avenues could be pursued and deepened in soil science, namely the exploration of:

(i) *in situ* diversity of the substrates (Song et al., 2020; Ahamed et al., 2023; Zheng et al., 2024), **a focus of this thesis**;

(ii) *in situ* enzyme properties, in terms of relative abundance and kinetics properties (Tian et al., 2020; Ghaderi et al., 2022; Khosrozadeh et al., 2022);

(iii) *in situ* intrinsic maximum growth rates of  microbial community (Flamholz et al., 2025; Foley et al., 2024).

Several empirical studies related to bioenergetics have also investigated microbial processing of C in soil using single substrates as organic inputs (e.g. Herrmann et al. 2014; Geyer et al., 2019; Bölscher et al., 2020; Endress et al., 2024). While adding single C substrates will further our mechanistic understanding of soil processes, this simplification is not accurately reflecting conditions in the soils where soil microorganisms are exposed to a very highly diverse organic matter.

## 3.2 Chemical composition of organic matter

Differences in organic matter inputs and composition of soil organic matter can arise from agroecosystem management, such as the application of organic amendments or the planting of different crop species or varieties, both in space and time. Increases in plant diversity have been shown to result in an increase in C storage in grassland soils (Lange et al., 2015; Spohn et al., 2023) and forest soils (Gamfeldt et al., 2013; Hulvey et al. 2013; Huang et al. 2018). These observations are in line with theoretical frameworks (Odum, 1969; Addiscott, 1995), i.e. that more complex ecosystems are more efficient in utilising resources, but they are often based on evaluating of changes in SOC over a longer period of time or they are using meta-analyses data sets. However, the underlying mechanisms by which plant diversity may enhance C storage remain unclear, particularly regarding how these effects are modulated by intrinsic soil properties, such as diversity of microorganisms, physical and chemical characteristics (McDaniel et al., 2016).

Tracking changes in SOC stocks in response to agroecosystem diversification typically requires several decades (Yi et al., 2025). As an alternative, intrinsic properties of the organic matter in the bulk soil may respond more rapidly, potentially serving as an early indicator of shifts in C content and its persistence (Lehmann et al. 2020).

It is known that different plant species and varieties, even within the same genus, can have diverging root traits that influence both the quantity and quality of carbon inputs to soil through litter and rhizodeposits (Smith 1969; Warembourg and Estelrich 2001; Pagès et al., 2014; Sun et al. 2017; Rees et al., 2020; Jiang et al., 2023). Moreover, a small proportion of root exudates is species- or variety-specific, whether between phylogenetically distinct plant species or among intraspecific varieties (Iannucci et al., 2017; Dietz et al., 2019; Bilyera et al., 2021; McLaughlin et al., 2023). Diversification of agroecosystems further alters the chemical diversity, composition and quantity of above- and below-ground organic inputs to the soil (El Moujahid et al., 2017; Mughal et al., 2024; Qiao et al., 2024), with corresponding modulations in the composition of microbial communities (Korenblum et al. 2022; Seitz et al. 2022), their metabolic products (Wiesenbauer et al. 2024), and their capacity to transform organic matter (Brolsma et al. 2017; Yergeau et al. 2014). However, it remains uncertain if such plant- and microbe-mediated differences are reflected in the composition of soil organic matter, especially when the changes arise from relatively subtle intraspecific varieties variations (Pérez-Izquierdo et al. 2018).

In addition to the composition of organic matter in the bulk soil, the properties of dissolved organic matter can sometimes be used as a proxy for estimating the persistence of soil organic matter (Tian et al., 2025). This is because soil solution or water-extractable organic matter respond rather rapidly to changes in agroecosystem management (Inselsbacher et al., 2011; Ohno et al., 2014; Randewig et al., 2019; Liptzin et al., 2022). Nevertheless, the molecular diversity of organic matter is often overlooked in studies and models of soil organic matter dynamics (e.g. German et al., 2011; Le Noë et al., 2023), despite a few exceptions (Hernández and Hobbie, 2010; Steinauer et al., 2016; Weverka et al., 2023).

The chemical properties of dissolved organic matter are equally heterogeneous as those of organic matter in the bulk soil (Swenson et al., 2015; Simon et al., 2025). Even though Orwin et al. (2006) stated that the chemical nature of substrate mixtures may have a greater impact on microbial activity than their diversity, they also suggested that greater organic substances diversity *decreases* microbial activity. The basis of this idea, i.e. a decrease in microbial activity with diversified organic inputs, is that processing a variety of compounds incurs higher metabolic costs compared to simpler mixtures or individual substrates (Lehmann et al., 2020; Nunan et al., 2020; Kothawala et al., 2021). This view is supported by experiments in lakes (Fonvielle et al., 2025) and has recently been translated into models (Weverka et al., 2023). However, the majority of studies suggests that higher substrate diversity *increases* microbial activity (Babel et al., 1993; Blagodatskaya et al., 2009; Okano et al., 2021; Tanentzap et al., 2019). According to this latter view, a broader array of organic compounds provides more ecological niches, allowing a larger proportion of the microbial community to participate in substrate processing (Loreau, 2001). The effects of chemodiversity may be influenced by interactions among the metabolic pathways involved in the processing of organic substrate mixtures, which can be either synergetic or antagonistic (Quigley et al., 2019). While both the chemodiversity and the intrinsic properties of the organic substrates could affect their processing by microorganisms, the importance of diversified organic inputs and their relative importance in possible synergistic or antagonistic interactive effects remains unclear.

## 3.3 Microbial energetic return on investment

In view of their low biomass in soil, microbial communities play a disproportionately important role in the soil C cycle (Miltner et al., 2012; Pausch et al., 2016; Wang et al., 2021b). As they are the primary agents of organic C transformation in the soils, it is therefore important to understand their functioning (Zheng et al., 2019; Martinez et al., 2024). As stated in the introduction, the term '*return on investment*' has been mentioned in the soil science literature as early as the beginning of this century (Schimel and Weintraub, 2003) but was not supported by empirical data when I began my doctoral studies. About a decade ago, Harvey et al. (2016) evaluated biodegradability of pyrogenic organic, and they proposed the quotient of the total energy content of organic substrates and their potential activation energy as an appropriate parameter as potential energetic ROI. Total energy was measured by bomb calorimetry whereas the potential activation energy was determined by stepwise isothermal thermogravimetric analysis. This intriguing paper made me curious, and I wondered whether this concept could be adopted in soils science.

The term '*return on investment*' originates from economics, and it is defined as the efficiency of investment, i.e. the ability to obtain the maximum benefit with the minimal input, avoiding waste of resources. Return on investment was adopted in microbiology as the flux of C, nutrients or electrons processed per unit of cellular resource invested (Basan, 2018; Bruggeman et al., 2020; Ni et al., 2020; Okano et al., 2021). Here, '*return on* *investment'* was evaluated by calculating the quotient between net benefit and direct cost of the microbial process. When considering processes related to the extraction of energy, the return on energy invested tends to be lower for more diffuse energy sources (e.g. solar radiation) than for more concentrated energy supplies (e.g. combustion of organic matter) (Jancovici and Blain, 2022). If return on investment decreases, the energy source may be more challenging to harvest. Since the extraction system uses a higher fraction of the potentially available energy, and a lower fraction remains for microbial work. In other words, there might be a positive (linear) relation between microbial activity and *'return on investment'*.

Soils are one of the most complex systems on earth in terms of their physical structure, chemical composition and biodiversity. Soil microbial communities face temporal and spatial variabilities of both biotic and abiotic factors impacting their access to chemically diverse organic matter (Lehmann et al., 2020; Ågren, 2021). Uncertainties regarding the quantity, quality, and delay of resource supply and costs associated with ecological interactions (i.e. competitors, cheaters, predators) can therefore have an impact on the efficiency of allocation processes (Allison 2012, 2014; Nunan et al., 2020). Evaluating bioenergetics in soils, such as accurately estimating the feasibility of enzymatic and microbial reactions or the amount of energy that microbial community can harvest from chemical gradients *in situ* over a given period is particularly difficult, even though tools have been developed to facilitate the calculations (Beber et al., 2022).

LaRowe and Van Cappellen (2011) proposed a simple approach, which approximates the nominal oxidation state of C (NOSC) by reducing the chemical structure of organic molecules to their elemental composition of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur (LaRowe and Van Cappellen, 2011). Across a wide range of organic compounds, they observed a negative linear relationship between the NOSC and $\Delta G^0_{\mathrm{Cox}}$ of these organic compounds (La Rowe and Van Cappellen, 2011 and equation (1) in section 4. Materials and Methods). Furthermore, the NOSC generally decreases in proportion to a potential activation energy of oxidation half-reaction, which can impact the kinetics of the reaction in a cellular context (Table 2). These relationships are based on a general trends between NOSC of functional groups and thermodynamic properties of substrates, i.e. the reduction potential, which explains the bioenergetics of redox reactions and helps to predict whether a reaction is energetically more favourable or less favourable (Weber, 2002; Bar-Even et al., 2012a, 2012b; Jinich et al., 2018) (see above 3.1 Bioenergetics in terrestrial systems). In summary, there is a potential to combine the approach of Harvey et al. (2016) and LaRowe and Van Cappellen (2011) to estimate the potential energetic return on investment (ROI) by calculating the quotient of the energy content of organic matter, using bomb calorimetry, with the potential activation energy on the assumption that $\Delta G^0_{Cox}$ can be calculated from the NOSC of organic matter.

For further, more detailed information on bioenergetics and return on investment, see 7. Appendix – Economic principles in soil microbial systems below.

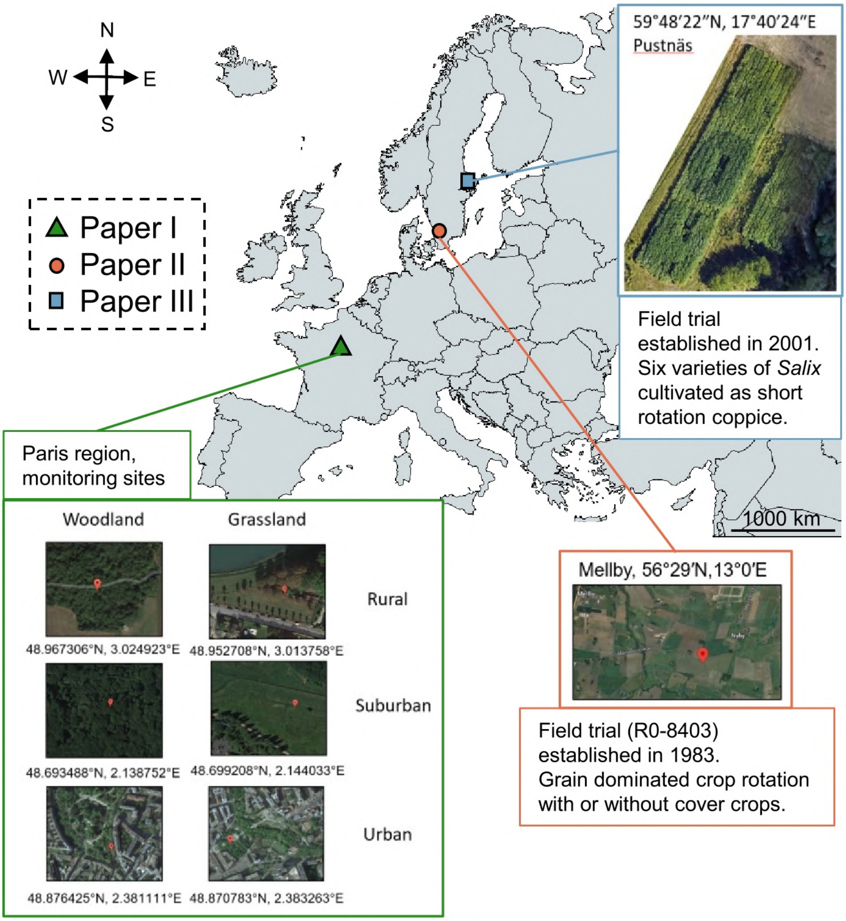
# 4. Materials and Methods

## 4.1 Soils

In this thesis, topsoil samples from various soil systems were used to investigate the influence of organic input properties on microbial activity and organic matter composition (Fig. 3). The soil samples were taken from (i) a range of grassland and woodland sites across an urban pressure gradient in the Paris region (**Paper I**), (ii) a grain-dominated crop rotations cultivated with or without cover crops in Mellby, South-West Sweden (**Paper II**) and (iii) a long-term field trials where *Salix* varieties were grown as short rotation coppice in Pustnäs, Uppsala, Central Sweden (**Paper III**) .

In **Paper I**, hot water-extractable organic matter (WEOM) was extracted from soils as it can be regarded as a proxy for the bioavailable portion of the soil organic matter (Guigue et al., 2014; Haddix et al., 2016). The extraction was carried out using pure water as a polar solvent at 60 °C for 30 min (Nkhili et al., 2012), then centrifuged at 4 °C for 10 min (5250 ×g). The supernatant was filtered at 0.7 µm and freeze-dried. The extraction procedure was chosen to reduce the alteration of organic matter, resulting in approximately 65% of hydrophilic compounds and 35% of hydrophobic compounds (Nkhili et al., 2012).

The three main research questions of the thesis (see section 2.) were investigated using a variety of approaches presented in the following sections.



Field trial established in 2001. Six varieties of *Salix* cultivated as short rotation coppice.

Field trial (R0-8403) established in 1983. Grain dominated crop rotation with or without cover crops.

**Figure 3**. Locations of sites for soil sampling.

## 4.2 Characterisation of the soil organic matter chemical composition

Hot WEOM and bulk soil organic matter were characterised using a range of analytical methods (Nebbioso and Piccolo, 2013; Chenu et al., 2024): (i) bomb calorimetry, (ii) ultrahigh-resolution mass spectrometry, (iii) pyrolysis-gas chromatography mass spectrometry (Pyrolysis-GC/MS), (iv) mid-infrared (mid-IR) spectroscopy.

### 4.2.1 Bomb calorimetry

For estimation of the potential energetic return on investment (ROI, see section 4.3 below), total energy of hot WEOM soil organic matter was determined as the heat of combustion (Harvey et al., 2016; Lorenz et al., 2024) using a Parr Oxygen Bomb Calorimeter 6300 M20609 (Parr instruments Moline, Illinois, USA) (**Paper I**). Benzoic Acid standardised for bomb calorimetry (Parr no. 3415, CAS.reg 65-85-0) was used as standard. Each sample's actual heat of combustion was then corrected for the external energy sources (cotton thread, ignition wire, benzoic acid). In this thesis, combustion enthalpy values were interchangeably used with specific energy content and presented as positive values for simplicity. Values were normalised by the organic C content of each organic matter sample and reported either as J $mmol^{-1}$ C ($\Delta E$) (**Paper I**), or kJ $g^{-1}$ C ($\Delta_C H$), to facilitate the comparison with other studies.

### 4.2.2 Ultrahigh-resolution mass spectrometry

The molecular composition and energetic potential of hot WEOM were determined by untargeted metabolomics (Hawkes et al., 2020; Bahureksa et al., 2021; Bhattacharjya et al., 2024) on a Bruker SolariX XR hybrid quadrupole Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR MS) (Bruker Daltonics, Bremen, Germany) (**Paper 1**). The analysis was carried out by direct infusion in the positive electrospray ionisation mode. For each sample, thousands of mass-to-charge ratios (m/z) were assigned to neutral molecular formulae using the TRFu algorithms (Fu et al., 2020) and then classified into biochemical classes using a multidimensional stoichiometric approach (Rivas-Ubach et al., 2018). The high sensitivity and resolving power of this method (Brown et al., 2024; Kim et al., 2022) allows the elemental composition of the molecules, which is necessary for the estimation of the NOSC (LaRowe and Van Cappellen, 2011). The NOSC can be estimated from the elemental composition using equation (1):

$$\text{NOSC} = 4 - [(4C + H - 3N - 2O + 5P - 2S) / C] \qquad (1)$$

where C, H, N, O, P and S refer to the intensity weighted stoichiometric number of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur atoms across molecular formula for each sample. The oxidation state of an atom is the charge of this atom after hypothesising that all heteronuclear bonds are ionic (Jurowski et al., 2015). The NOSC in molecular formulae also represents the average number of valence electrons linked with C atoms (Flamholz et al., 2025). A NOSC of 0 indicates neutrally-charged C (4 valences electrons), negative or positive NOSC indicates respectively an excess or a deficit of electrons compared to the neutrally-charged C (Flamholz et al., 2025).

### 4.2.3 Pyrolysis-GC/MS

Organic matter of dry and milled bulk soil (**Paper III**) was analysed by Pyrolysis-GC/MS in the presence of tetramethylammonium hydroxide (TMAH 25% methanol). First, pyrolysis, which is the heating of organic matter in an atmosphere free of dioxygen, occurred in a pyroprobe (6250, CDS) and lead to thermolysis. Then, pyrolysis was coupled with chromatography and a mass spectrometer to characterise at a high resolution soil organic matter composition. Variations in the thermal energy supplied during the pyrolysis process influenced the dynamics of bond cleavage. The major consequence of pyrolysis was the fragmentation of large compounds into smaller ones (Stewart, 2012), which allowed larger molecules to be more volatiles because they had lower molecular masses. The pyrolysate, which had a relatively lower polarity and is present as a gas, was injected into a gas chromatograph (7890B, Agilent) coupled to a quadrupole mass spectrometer (5977B, Agilent). Specifically, in the gas chromatograph, a non-polar column was used Rxi-5Sil MS (30 m × 0.25 mm × 0.5 µm, Restek). The ionisation technique used was electron ionisation. The classification of pyrolysis products was carried out based on previous works (Derenne and Quénéa, 2015; El Hayany et al., 2021; Vidal et al., 2016). The molecular diversity of bulk soil organic matter was characterised by the effective Simpson index of pyrolysis products in **Paper III**, or as the Shannon diversity index, to facilitate the comparison with other studies.

### 4.2.4 Mid-IR spectroscopy

Dry and milled bulk soils (**Paper III**) were analysed in the laboratory by Fourier transform mid-infrared (FTIR) diffuse reflectance spectroscopy (DRIFT) (Guillou et al., 2015; Pärnpuu et al., 2022; Wetterlind et al., 2022). The Savitzky-Golay function was applied on each spectrum to smooth the data (Savitzky and Golay 1964). In order to correct the potential light scattering, a normalisation step was done by standard normal variate (Morais et al., 2020). The full mid-IR spectral range (4000 $cm^{-1}$ to 630 $cm^{-1}$ or 2500 nm to 15,873 nm) was used as inputs for multivariate statistics (Ramírez et al., 2021). Different regions of the spectra were assigned to functional groups observed in organic molecules (Parikh et al., 2014; Sharma et al., 2021).

## 4.3 Potential energetic return on investment

The potential energetic return on investment (ROI, **Paper I**) that chemoorgano-heterotrophic microbial communities can potentially extract during the transformation of the WEOM in soil was calculated as follows:

$$\mathrm{ROI} = \Delta E / \Delta G^0_{\mathrm{Cox}} \quad (2)$$

where both $\Delta E$ and $\Delta G^0_{\mathrm{Cox}}$ are in J $mmol^{-1}$ of C. $\Delta E$ is determined by bomb calorimetry (section 4.2.1) and is the maximum potential energy that can be harvested by chemoorganoheterotrophic microbial communities when processing diverse organic matter in aerobic condition. i.e. in the absence of any metabolic constraints, such as e.g. in activity hotspots. $\Delta G^0_{\mathrm{Cox}}$ represents the change in standard Gibbs free energy of oxidation half-reaction of the organic matter and was estimated by LaRowe and Van Cappellen (2011) as follows:

$$\Delta G^0_{\mathrm{Cox}} = 60.3 – 28.5 \times \mathrm{NOSC} \quad (3)$$

$\Delta G^0_{\mathrm{Cox}}$ is derived from the NOSC and determined by ultrahigh-resolution mass spectrometry (section 4.2.2). High $\Delta G^0_{\mathrm{Cox}}$ reflects low thermodynamic favourability in an electron donor half reaction occurring in standard conditions (at 25 °C, 100 kPa) where organic matter is oxidised (Graham et al., 2023). This lower thermodynamic favourability is assumed in the present thesis to be associated with higher cellular expenditure to harvest energy in a cellular context (Jinich et al., 2018).

## 4.4 Characterisation of the soil microbial composition

Microbial communities in soils were investigated through (i) their taxonomic composition with metabarcoding and (ii) their activity using isothermal calorimetry.

### 4.4.1 Microbial community composition

The composition of the soil bacterial community was determined (**Paper I**). In summary, soil DNA was extracted, the V3–V4 DNA regions encoding for the 16S rRNA sequences were first amplified and then the PCR products were analysed through Illumina Miseq sequencing (2*250 bp). These pre-processed sequences were then used as input to the DADA2 pipeline (Callahan et al., 2016) to generate amplicon sequence variants (ASVs). ASV sequences were classified taxonomically. The abundance table of ASV and the bacterial ASV sequences were used as input to the web-based server MicFunPred (http://micfunpred.microdm.net.in/), which minimises false-positive results in comparison to other approaches (Mongad et al., 2021). This allowed to estimate the predicted number of 16S rRNA gene copies per bacterial genus. Based on the relative abundance table for each genus in the soil samples, the weighted average number of 16S rRNA gene copies for each soil bacterial community were estimated.

### 4.4.2 Isothermal calorimetry

Microbial activity was investigated by measuring heat production after adding organic material (von Stockar and Liu, 1999; Kemp, 2000; Lemos et al., 2019). The data was used to investigate potential relationships with properties of (i) low molecular weight water-soluble soil organic matter (NOSC, C-to-N ratio, molecular size and diversity) (**Paper II**), (ii) mixtures of small and large molecules and colloids, such as molecules with a molecular weight below 1kDa in combination with molecules and colloids larger than 1 kDa (enthalpy of combustion, return on investment), and (iii) bacterial communities composition (16S rRNA gene copies) (**Paper I).** Heat dissipation rates were determined over 1 day in isothermal calorimeters (TAM AIR, Sollentuna, Sweden) at 25°C (**Paper I**) or at 15°C (**Paper II**). In **Paper I**, the soils were incubated prior to the calorimetric measurement for 4 days at 25 °C at a matric potential of − 0.033 Mpa. In **Paper II**, the soils were incubated for 10 days at 15°C and 45% maximum soil water holding capacity to standardise soil conditions. Then, aliquots of 5 g soil (dry weights) were placed in 22 ml glass reaction vial and amended with (i) 300 µg $C_{org}$ $g^{-1}$ soil (i.e. mixture of small and large molecules) (**Paper I**), or (ii) 50 µg C $g^{-1}$ soil of either 18 individual substrate additions (i.e. low molecular weight C substrates, molecular richness = 1) or their combinations resulting in mixture richness of 2, 4, 6, 9, 12 or 17 (**Paper II**). The soil heat dissipation of the added organic inputs was determined by subtracting the heat dissipation in the milli-Q water treatment. In **Paper II**, heat dissipations from individual substrates were used to calculate theoretical heat dissipation values of the various substrate diversity mixtures, and theoretical and experimental heat dissipations were compared.

# 5. Results and Discussion

## 5.1 Below-ground organic matter properties and diversified organic inputs to soil

This section focuses on research question 1: Specifically, what is the impact of diversified organic inputs to soil on the (i) molecular diversity, (ii) energy content, (iii) elemental stoichiometry, (iv) molecular composition or mass of soil organic matter (**Papers I and III**). Soil organic matter is a continuum of organic matter transformation with more advanced degree of transformation in mineral-associated versus particulate organic matter, and in soil compared to litter, plant residues (Williams et al. 2018; Lorenz et al., 2024). It is therefore to be expected that soil organic matter has lower C-to-N ratios, lower energy contents, higher NOSC values, an intermediate molecular mass and a less diverse community of molecules compared to litter and plant residues.

### 5.1.1 Chemical composition of organic matter extracted from woodland and grassland soils

In **Paper I**, the combustion enthalpies and the weighted means of NOSC were lower in the WEOM from woodland compared to WEOM derived from grassland soils (Table 3). However, the weighted mean ratios of C-to-N of WEOM were higher in woodland compared to grassland WEOM (Table 3). In respect to molecular richness, WEOM was similar in grassland and woodland soils (Table 3). Furthermore, the relative abundances of each compounds class (i.e. phytochemicals and oxy-aromatic compounds, lipids, unknown, peptides, amino sugars and carbohydrates) was the same in grassland and woodland soils (Table 4).

**Table 3.** Properties of organic matter. The mean ± one standard deviation or the range from the minimum to maximum values are depicted. Abbreviation: water-extractable organic matter (WEOM).

| References | Organic matter | Combustion enthalpies (kJ per g C) | C oxidation state (intensity-weighted average) | C-to-N ratio (intensity-weighted average) | Molecular mass (intensity-weighted average) | Molecular richness | Effective Simpson´s diversity index |
|---|---|---|---|---|---|---|---|
| **Paper I** | All samples | 45.2 to 56.0 | -0.47 to -0.16 | 7 to 11 | | 1896 to 2147 | 27 to 55 |
| | Woodland WEOM (n=3 except for combustion enthalpies where n= 2) | 45.2 to 47.3 | -0.40 ± 0.09 | 10 ± 0.8 | 384 ± 39 | 2017 ± 126 | 38 ± 13 |
| | Grassland WEOM (n=3) | 50.0 ± 5.3 | -0.24 ± 0.09 | 8 ± 0.8 | 408 ± 14 | 1968 ± 49 | 46 ± 9 |
| **Paper II** | Single substrate | 10.0 to 62.4 | -1 to +3 | 2 to 9 (+ molecules without N) | 68 to 973 | 1 | 1 |
| | Substrate mixtures | 21.9 to 50.4 | -0.8 to 1.6 | 4 to 32 (+ cocktails without molecules containing N) | 75 to 276 | 2; 4; 6; 9; 12; 17 | 2 to 17 |
| **Paper III** | *Salix viminalis* (0-20 cm topsoil in the fertilised treatment) (n=4) | | | | | 132 | 6 ± 1 |
| | *Salix dasyclados* (0-20 cm topsoil in the fertilised treatment) (n=2) | | | | | 132 | 11 ± 2 |

**Table 4.** Molecular composition of organic matter in different soils. FT-ICR MS was used in **Paper I** and pyrolysis-GC/MS was applied in **Paper III**. The mean ± one standard deviation is depicted. When n=2, the range from the minimum to maximum values is depicted. Biochemical categories and most probable origin are based on Vidal et al. (2016) and Rivas-Ubach et al. (2018).

| Most probable origin | Biochemical categories | Woodland WEOM (n=3) % | Grassland WEOM (n=3) % | *Salix viminalis* (0-20 cm topsoil in the fertilised treatment) (n=4) % | *Salix dasyclados* (0-20 cm topsoil in the fertilised treatment) (n=2) % |
|---|---|---|---|---|---|
| | | **Paper I** | **Paper I** | **Paper III** | **Paper III** |
| Multiple origins | Phytochemical and oxy-aromatic compounds | 39.0 ± 3.5 | 41.7 ± 1.7 | | |
| | Lipids | 26.5 ± 2.3 | 23.6 ± 1.3 | | |
| | Not Matched | 18.2 ± 0.9 | 18.8 ± 0.9 | | |
| | Peptides | 12.4 ± 1.6 | 13.0 ± 0.4 | | |
| | Amino sugars | 2.1 ± 0.5 | 1.6 ± 0.2 | | |
| | Carbohydrates | 1.7 ± 0.5 | 1.1 ± 0.2 | | |
| | N compounds, other N-heterocyclic compounds, carbohydrates, phenols, benzene derivatives, polyaromatic compounds, and other aliphatics | | | 80 ± 4 | 78 |
| Plant-derived | lignin, long-chain fatty acid, cutin and /or suberine originated compounds, long-chain alkane | | | 15 ± 4 | 17-18 |
| Microbial-derived | Short-chain fatty acid, short-chain alkane | | | 5 ± 1 | 4-5 |

In **Paper I,** soil organic matter in grassland soils was more labile and of better quality than the organic matter in woodland soils, which is consistent with published literature (Chantigny, 2003). Additionally, differences in the stoichiometry of organic matter inputs may be partly reflected in the stoichiometry of WEOM: Woodland WEOM had a higher C-to-N ratio compared to grassland WEOM, very likely due to organic inputs rich in lignin etc., and soils receiving organic matter originating from woodland may have been slightly more N-limited than those from grasslands. The molecular masses of WEOM did not differ between woodland and grassland soils (Table 3) (**Paper I**). This may seem surprising, given that Lange et al. (2021) found that diversification in grasslands decreased the mean molecular weight of dissolved organic matter (Lange et al., 2021).

Furthermore, the results of **Paper I** are in support with the results by Lorenz et al. (2024): WEOM extracted from grassland and woodland soils had an energy content between 45.2 to 56.0 kJ per g C (**Paper I**), which is 1.25 up to 2 times higher than energy content determined in organic and mineral horizons as well as particulate organic matter derived from forest soils. Results are also in line with Lange et al. (2021), who found that diversification in grassland had an effect on the oxygen-to-carbon ratio and degree of reactivity of small molecules in soil organic matter.

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

In the long-term *Salix* field trial at Pustnäs, Uppsala, Sweden, field replicates of the unfertilised treatment showed high spatial variability, particularly in pH, calcite ($CaCO_3$), total N and organic C contents, and no differences in chemical composition of soil organic matter were observed in both the pyrolysis GC/MS and mid-IR methods (**Paper III**). In contrast, in the nutrient fertilised treatment, differences in molecular composition of soil organic matter were observed at the *Salix* species rather than variety level. The results from **Paper III** are consistent with recent findings that soil organic matter under the Loden *Salix* variety (*S. dasyclados*) had, on average, higher molecular diversity than the Tora *Salix* variety (*S. viminalis*) (Table 5 and Jensen et al., 2024). Jensen et al., (2024) examined molecular diversity of soil organic matter of sites located along a 1000 km latitudinal gradient. These studies provide evidence that the identity of *Salix* species or varieties can affect the molecular composition and diversity of soil organic matter.

Corresponding traits should be considered in *Salix* species to enhance the organic C accumulation and persistence in the soils that these *Salix* species are grown on. In future studies, intra-cropping i.e. growing different varieties together, would be interesting to determine whether these results are maintained or amplified in diversified systems.

**Table 5.** Composition and amount of above-ground biomass and composition of pyrolysis products from bulk soil (**Paper III** and Jensen et al., 2024*).

| | *Salix viminalis (n=4)* | *Salix dasyclados (n=2)* |
|---|---|---|
| Lignin relative abundance (%) in bulk SOM (0-20 cm) (**Paper III**) | 11± 1 | 14-15 |
| Lignin (% of total solids content) [!] in biomass of shoot (bark+wood) | 25.8 ±1.2 | 28.4 (27.9-28.8) |
| Accumulated shoot C (t per ha) [“] | ≈ 80 to 120 | ≈ 45 to 55 |
| Root colonisation by *Cortinarius* spp. [&] | No | yes |
| Sodium-to-carbon (Na-to-C) ratio in leaves biomass ^ | 36 | 163 |
| Shannon diversity index (H´) of SOM at the Rostock site* | 5.82 | 6.71 |
| Shannon diversity index (H´) of SOM at the Uppsala site* | 13.36 | 14.66 |
| Shannon diversity index at the Pustnäs site in the fertilised treatment (**Paper III**) | 2.70 ± 0.14 | 3.02-3.08 |
| Shannon diversity index at the Pustnäs site in the unfertilised treatment (**Paper III**) | 2.61 ± 0.29 | 2.60-2.62 |

Values are means or minimum-maximum range. [!]Above-ground lignin content data are from Kalita et al. (2023). “Accumulated shoot C for 17 years data are from Rönnberg-Wästljung et al. (2022) and Baum et al. (2020). [&]Data about the colonisation of roots by fungi (n=1) are from Püttsepp et al. (2004). ^Data about sodium-to-carbon ratio (n=3 for *Salix viminalis* and n=2 for *Salix dasyclados*) are from Ågren and Weih (2012). *Shannon diversity indexes of soil organic matter (n=1) at the Rostock and Uppsala sites are from Jensen et al. (2024). Soil organic matter pyrolysis data and Shannon diversity index of soil organic matter at the Pustnäs site are presented in **Paper III**.

## 5.2 Soil microbial responses to diversified organic inputs

The following section focuses on the research question 2: How do diverse organic inputs to soils affect microbial activity? (**Papers I** and **II**)

### 5.2.1 Hot water-extractable organic matter and microbial activity

There were clear differences in the dynamics of heat dissipation due to microbial activity among soils and organic matter inputs provided in the form of WEOM (Fig. 2 in **Paper I**). The overall shape of the heat dissipation curves dynamics were dependent on the soil. Within each soil, heat dissipation was generally highest in soil amended with urban or rural grassland WEOM and lowest when soils received rural or suburban woodland WEOM. Overall, the data presented in **Paper I** suggested that the dynamics of organic matter consumption is more related to the taxonomic composition of bacterial communities than the composition of WEOM. The soils with more rapid heat dissipation dynamics harboured higher relative abundances of *Bacteroidia* and *Gammaproteobacteria*, both of which are recognised to contain many copiotrophs (Fierer et al., 2007; Shrestha et al., 2007). *Alphaproteobacteria* and *Actinobacteria*, both of which were relatively abundant in the soil with the slowest heat dissipation dynamics, are known to be dominated by phylotypes with low 16S rRNA operon copy numbers (DeAngelis et al., 2015; Shrestha et al., 2007). That microbial communities are a critical component in the terrestrial C cycle is generally acknowledged. The challenge ahead is to disentangle the interactions between organic matter and the composition of microbial communities in soils (see also below 5.3 Potential energetic return on investment (ROI) and activity of soil microbial communities).

### 5.2.2 Molecular diversity of organic input on microbial activity

Cumulative heat dissipation rates did not correlate with the molecular richness of the substrate mixtures added to soils (see Fig. 1 of **Paper II**) or molecular formulas of WEOM derived from FT-ICR MS (see Fig. 4a below) (**Paper I)**. These results did neither support the view of Orwin et al. (2006) that substrate diversity *decreases* microbial activity nor the view that substrate diversity *increases* microbial activity (Babel et al., 1993; Blagodatskaya et al., 2009; Okano et al., 2021; Tanentzap et al., 2019). The range of substrate diversities added to soils in **Paper II** was relatively low compared with the chemical diversity of organic matter in soils, which could be an explanation that molecular diversity of organic matter did not have an effect on microbial activity. Another explanation could be that microbial activity is usually restricted to soil environments that usually contain low amounts of organic matter (Allison et al., 2014; Lehmann et al., 2020; Kothawala et al., 2021). The amounts of C inputs to soils used in **Papers I and II** were 300 or 50 μg C per g soil, respectively. Consequently, C concentrations added to the soils in my studies were much higher than those typically found in soils (e.g. Gunina et al., 2017), and the expression of extracellular enzymes and transporter proteins was probably justified in my studies as there was always a sufficient energetic return.

Furthermore, when adding distinct low molecular weight organic compounds with known chemistry as a diversity gradient of substrate mixtures to soil, total heat dissipation displayed a bell-shaped pattern in relation to NOSC (see Fig. 2 in **Paper II**). The fact that microbial activity is low at both ends of the NOSC scale can be explained as follows: Oxidised organic matter with positive values of NOSC have fewer electrons per C, and microorganisms need to allocate a larger portion of co-factors to activate their half-reactions of reduction (Jinich et al., 2018); on the other hand, microorganisms exposed to reduced organic matter with negative values of NOSC and more electrons per C may produce an excess of ATP (Flamholz et al., 2025), and this cytotoxic effect of excess ATP would need to be balanced (Pontes et al., 2016). The bell-shaped relationship between NOSC and heat dissipation was not evident when adding WEOM to soils (see Fig 5a below), but heat dissipation was negatively correlated with the average of C-to-N ratio of small molecules (<1 kDa) (Fig. 4b below). In **Paper II**, I added low molecular weight organic compounds with known chemistry, while in **Paper I**, WEOM was extracted from soils with unknown chemistry, here FT-ICR MS was used for characterisation of this organic material and NOSC and other properties were inferred. The addition of WEOM is closer to reality, but in **Paper I** the C concentration of WEOM was six times higher than the C concentrations added in **Paper II**. In the future, it would be interesting to evaluate the relationship between NOSC and microbial activity in relation to dissolved organic matter that has a similar C concentration to soils.

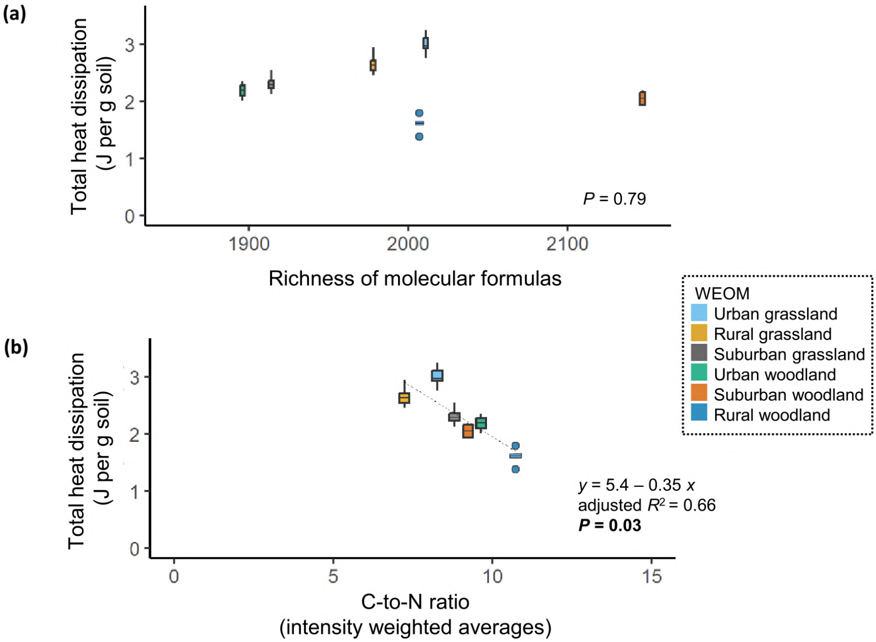


**Figure 4**. Relationships between total heat dissipation and (**a**) richness of molecular formulas (< 1 kDa) (**Paper I**) and (**b**) the intensity-weighted averages C-to-N ratio of small molecules (<1 kDa) (**Paper I**). Boxplots show (i) the median as crossbars inside boxes, (ii) 75th and 25th percentile as crossbars on the top and the bottom of boxes respectively, (iii) the 99th and 1st percentile as error bars above and below the boxes respectively (n = 6 soils, after averaging the quadruplicate of the same treatment during the isothermal calorimetry analysis).

In general, the measured overall heat dissipation in response to inputs of diversified substrate mixtures were higher than the estimated values, the latter was calculated from the weighted sum of heat dissipated from individual C substrates (**Paper II**). At relatively *low* molecular richness, *positive interactive effects* of C substrates reflect the synergetic outcomes when a microbial community processes complementary organic compounds (**Paper II**). These observations are consistent with the view of an auxiliary substrate framework suggested by Babel (2009): The framework suggests that the presence of a specific substrate in a mixture can sometimes provide additional co-factors once processed and therefore enhance some microbial traits. In **Paper II**, the C substrate formate is used together with glucose (Gommers et al., 1988) or formate is used with glycine, an amino acid containing nitrogen. Enhanced nitrogen availability (Koranda et al., 2014) may be the cause of positive interactive effect when formate and glycine are added to soils simultaneously. However, geochemical models (e.g. Quigley et al., 2019) usually assume that organic substrates are processed additively, i.e. each independent pool of organic matter is transformed at a given rate. At *higher* molecular richness, there is *no interactive effect* possibly due to more complex processing of organic substances by microorganisms. Here, an upper limit of microbial activity could generally be estimated from the highest measured microbial activity when supplied as individual substrate (preferential use). The preferential use observed in **Paper II**, however, is compatible with the geochemical view, and further investigations are required to constrain microbial- and substrate-explicit models (Zheng et al., 2024) of soil organic matter dynamics.

## 5.3 Potential energetic return on investment (ROI) and activity of soil microbial communities

This section focuses on research question 3: How does the potential energetic ROI that the microbial community can get from processing organic matter affect their overall activity in soil? The working hypothesis associated with this research question was that the potential energetic ROI correlated positively with overall soil microbial activity. Overall, this hypothesis was confirmed (Fig. 5c, **Paper I**). Our results suggest that microbial communities preferentially use organic matter with a high potential energetic return on investment, i.e. organic molecules that do not require high cost associated with catalysis whilst yielding a high net energetic benefit.

The overall heat dissipation from soils was not directly related to the NOSC of the WEOM (Fig. 5a) (**Paper I**). In a variety of ecosystems ranging from arable land, woodland, peatland and sediments from lakes and coastal sites, Vaziourakis et al. (2025) identified a significant, linear positive correlation between the total energy content of the organic matter in bulk soils or sediments and microbial activity. This relationship was also observed in the present thesis ($R^2 = 0.73$ and $P = 0.42$; Fig. 5b *vs.* $R^2 = 0.76$ and $P = 0.024$ in Vaziourakis et al., 2025). When calculated the potential energetic ROI, the relationship to microbial activity was significantly improved, but had a negative intercept that did not follow a 1:1 relation (Fig. 5c). This suggests that some of the soil organic matter was not prone to decomposition. The potential energetic ROI values in **Paper I** ranged from 7.4 to 10.0. Harvey et al. (2016) reported ROI values for pyrogenic organic matter between 0 up to 3.5; in the study of Williams and Plante (2018), ROI ranged from 0.07 to 0.33, and (iii) for topsoil versus sub-soil ROI was on average 1.10 ± 0.03 0.78 ± 0.03, respectively (Henneron et al. (2022). Rovira et al. (2008) also analysed from a similar energetic perspective (e.g. benefit-to-cost ratio) contrasting plant litters and linked this index with biochemical parameters of the decomposing litter.

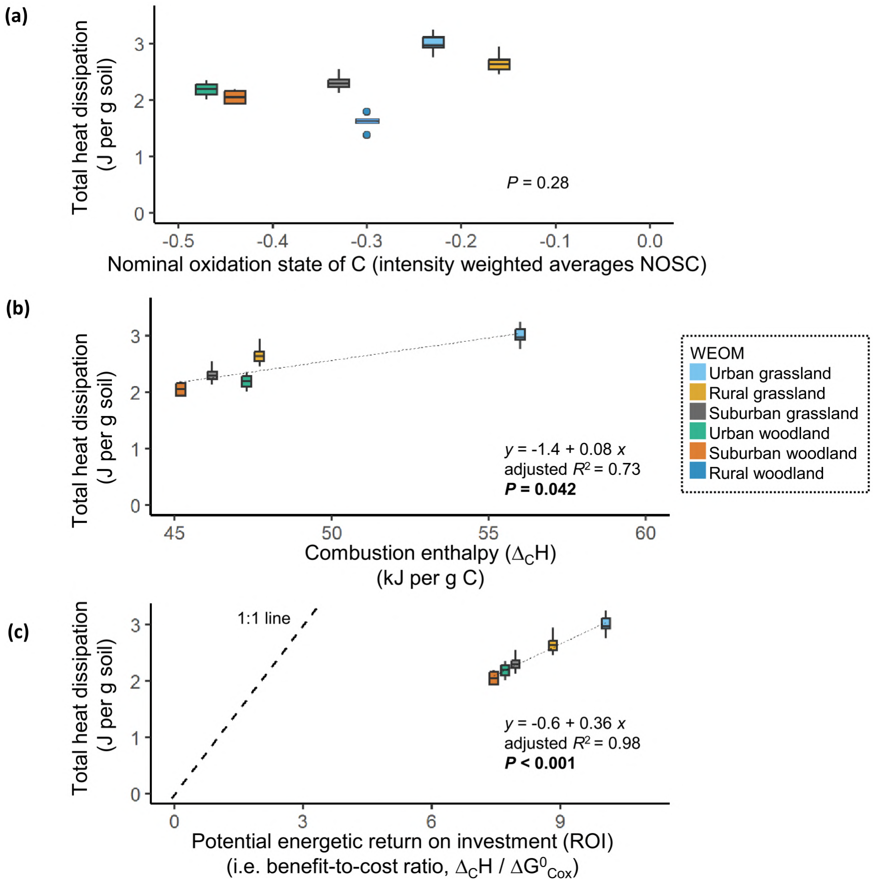


**Figure 5**. Relationships between the overall heat dissipation (n = 6 soils, after averaging the quadruplicates of the same treatment during the isothermal calorimetry analysis) and (**a**) the nominal oxidation state of C (ion intensity-weighted averages NOSC) of small organic substances (<1000 Da) in water-extractable organic matter (WEOM), (**b**) the combustion enthalpy or specific energy content of the bulk WEOM ($\Delta E$, in **Paper I**), and (**c**) the potential energetic return on investment (ROI) associated with the microbial processing WEOM by assuming differential processing of small and large molecules.

I must admit that the methods used to determine and evaluate ROI in the above-mentioned studies were different from the method used in the present thesis. Furthermore, the type of organic matter varied considerably across studies (pyrogenic organic matter *vs.* labile, readily available organic matter etc.), so a direct comparison may not be appropriate or sufficient. It should be taken into account that **Paper I** explored the decomposition of WEOM, a labile, readily available organic material added at relatively high concentrations. Therefore, the potential energetic ROI maybe a relevant indicator for predicting microbial activity in hot spots, e.g. in the rhizosphere. The question remains whether potential energetic ROI can be related to microbial activity in low-energy areas such as e.g. the bulk soil. If this (linear) relationship persists, ROI has the potential to be included as model variable in novel simulation models taking into account microbial energy limitation (Wutzler and Reichstein, 2013; Coucheney et al., 2025)

## 5.4 Method comparison: Pyrolysis-GC/MS and mid-IR spectroscopy

**Paper III** included a method comparison between the pyrolysis-GC/MS method and the mid-IR spectroscopy approach. Overall, in the nutrient fertilised treatment, the *Salix* variety Gudrun and, to a lesser extent, Loden were separated from the other varieties (see **Paper III** Fig. 4b) using pyrolysis-GC/MS. The separation was due to higher abundance of phenol derivatives of unspecific origin. Furthermore, Gudrun was enriched in nitrogenous compounds from proteins and nucleic acids compared to Tordis and Tora. The composition of soil organic matter below *Salix* varieties Loden and Jorr differed from the other four varieties (Paper III, Fig. 3b) when mid-IR was used to characterise soil organic matter. Even though each method allowed separating the influence of different *Salix* varieties on the composition of organic matter in the bulk soil, a Mantel test showed that the two methods were not closely related. In other words, the two methods discriminated certain varieties from others, but not the same varieties. The choice of which method is most suitable depends on the question to be addressed. The mid-IR spectroscopy is a simpler, cheaper and has the advantage of being non-destructive compared to other approaches, such as pyrolysis-GC/MS approach (Sanderman et al., 2020; Margenot et al., 2023) or nuclear magnetic resonance (NMR) (Shi et al., 2022; Chakrawal et al., 2024). In contrast, the pyrolysis-GC/MS approach offers a more detailed analysis, allowing the pyrolysis products to be classified into their potential original molecular groups, such as from plant or microbial origin. Pyrolysis GC/MS could be a good compromise between high-throughput mid-IR spectroscopy and more advanced methods such as NMR or FT-ICR MS.

# 6. Conclusions and future perspectives

*"If we begin with certainties, we shall end in doubts;*
*but if we begin with doubts, and are all patient with them,*
*we shall end with certainties."*

De Augmentis Scientarium, Francis Bacon

Soils provide the largest store of terrestrial C, and they play a central role in achieving sustainable development. The notion of changing agricultural practices to increase belowground C storage is gaining scientific, economic and political credence, and the EU Common Agricultural Policy reform acknowledges this challenge and promotes a policy of sustainable intensification of agricultural land e.g. via crop diversification. This thesis examined the extent to which the diversity of organic inputs influences soil C dynamics, especially through its interactions with microbial communities. Overall, I raised the question: *Does the diversity of organic inputs matter for C dynamics in soils*? The short answer is "it depends".

The potential energetic return on investment was positively correlated with soil microbial activity when amended with various dissolved organic matter (**Paper I**). This linear relationship suggests that this term could be included as a variable in mechanistic models of soil organic matter describing e.g. microbial limitation. However, the soil environment in this study represented the rhizosphere with its C hot spots rather than the bulk soil where microorganisms are often C limited. Future studies should therefore explore the relationship between potential energetic return on investment and microbial activity under C limited conditions. From a mechanistic perspective, it would be interesting to investigate the relationship between return on investment and microbial physiological properties such as e.g. microbial C use efficiency, enzyme activities etc.; possible relationships still in need to be explored.

Microbial activity in response to diversified organic inputs was dependent on their molecular richness: At very low molecular richness, interactive effects of C substrates were revealed; at higher molecular richness, no interactive effects were observed (**Paper II**). In the latter case, microbial communities process organic substrates additively according to preference order, and these situations resemble those in the soil environment. Outcomes of this study provide information for the further development of, e.g., microbial- and substrate-explicit mechanistic models.

*Salix* species, but not varieties, had an influence on the composition of soil organic matter and diversity (**Paper III**). Changes in chemical composition of organic matter were subtle but deteactable by pyrolysis GC/MS and mid-IR spectroscopy. This study focused on individual *Salix* species and varieties. Future studies could explore "intra-cropping", i.e. growing different species and varieties together, to investigate whether these results are maintained or amplified in diversified systems.

Soil microorganisms are the engine of the Earth, responsible for many of the key ecosystem services which soils deliver. They require energy and nutrients from the decomposition of organic matter. Soils are complex and the challenge ahead remains that soil scientists need to simplify and make assumptions when exploring soil processes. In respect to diversified organic inputs, soil C cycling is best understood through the interactions between organic matter properties and the microbial communities that process organic matter, rather than through the lens of input diversity of organic inputs alone.

# 7. Appendix – Economic principles in soil microbial systems

Among the requirements for life are nutrients, C and redox gradients from which energy can be harvested. Microorganisms, as autopoietic systems, must allocate their cellular activities in efficient ways to extract and process resources from their environment to renew their cellular structures and machinery (Fleischaker and Margulis, 1986). This is associated with the dissipation of a minimum amount of thermal energy (Saadat et al., 2020). These cellular activities includes motion to access substrates (Ni et al., 2020), modulation of cellular size (Harris and Theriot, 2018; Belliveau et al., 2021), and the expression of transporters and enzymes to process substrates. For instance, larger or more abundant enzymes may be required for energetically demanding reactions (Arcus et al., 2016; Davidi et al., 2016, 2018). The way microorganisms allocate resources into different cellular functions (Belliveau et al., 2021; Scott and Hwa, 2023) and the kinetics and thermodynamics of microbial reactions, such as redox biochemical reactions (Harold, 1987; Bar-Even et al., 2012a, 2012b; Noor et al., 2012, 2013, 2014; Davidi and Milo, 2017) are two perspectives that could explain some aspects of the functioning of microorganisms as part of a population (Flamholz et al., 2025). However, additionnal microbial activities may need to be considered in a soil context, where microbial community need (i) to access substrates in remotes pores or particle/aggregate surfaces through motility (Ni et al., 2020) and (ii) produce substances involved in cross-feeding, required in the formation of biofilms, internal storage compounds or in solubilisation processes (Kreft, 2004; Li et al., 2021, Mason-Jones et al., 2022).

## 7.1 Return on investment as a guiding but constrained principle

### 7.1.1 Return on investment as a guiding principle for microbial resource allocation

From a cybernetic system perspective, microorganisms function as goal-seeking self-regulating entities that convert energy and replicate themselves (Ramkrishna and Song, 2012). They allocate their proteome strategically to maximise their fitness, balancing trade-offs, such as between rates and efficiency of growth, motility and long-term survival (Wilken et al., 2021; Flamholz et al., 2025). This is why microorganisms generally follow economic principles (Milo and Phillips, 2015; The Economic Cell Collective, 2024). The way microorganisms manage to allocate their cellular machinery is generally similar to lean production through the minimisation of wastes and costs, and the maximisation of quality and productivity (Basan, 2018; Liu et al., 2024). Basan (2018) stated that “lean production pathways maximise growth rate by maximising the return on investment of finite cellular resources”. Some metabolic pathways, like fermentation, may appear like a wasteful allocation strategy because they have a relatively lower return than respiration, in terms of ATP yield. Yet, the lower requirements of fermentation pathways for enzyme investment compared with respiration make fermentation a widespread strategy (Basan, 2018). Furthermore, when a preferred C source is accessible, there is a reproductive fitness advantage of investing cellular resources in processes such as motility and chemotaxis to then uptake and transform the compound, which generally translates into the maximisation of the potential return, such as higher growth rate (Ni et al., 2020).

The higher the investment into a cellular function is, the higher the potential fitness of microorganisms. However, it remains unclear whether the correlation between the investment in cellular function and the potential benefit associated with is causal or predictive (anticipatory) (Ni et al., 2020). In the case of microbial metabolism, lower returns, such as slower growth rates, lead microorganisms to allocate more proteins to transporters and enzymes in peripheral and catabolic pathways, rather than in biosynthetic and polymerisation pathways (Basan et al., 2015; Basan, 2018). On the contrary, the relationship between the potential return of chemotaxis and the investment in motility is predictive (Ni et al., 2020). This means that microorganisms may sometimes pre-invest into cellular functions in proportion to the predicted future benefit (Ni et al., 2020) as insurance to cope with highly variable nutritional conditions that often occur in soil systems (Loreau et al., 2021; Mori et al., 2021). An example of this less efficient way to allocate cellular resources in the short-term is the maintenance of inactive ribosomes to varying levels depending on the microbial taxa, which can partly explain the temporal microbial activity response to the availability of resources (Placella et al., 2012).

The return on investment is supposed to constrain the way substrates are taken-up, either sequentially or simultaneously, when considering the question of nutritional preferences between microorganisms (Okano et al., 2021; Weverka et al., 2023). In particular, substrates, when taken up, have different entry points in the central C metabolism and they may be processed simultaneously because the return on investment would favour it (Wang et al., 2019; Okano et al., 2021). Yet, optimal resource allocation appears to be driven by microbial preferences that are encoded in the genetic program that results from trade-offs in response to evolutionary and ecological factors rather than physicochemical properties of resources (i.e. energy content of organic substrates) (Aidelberg et al., 2014; Hermsen et al., 2015; Wang et al., 2019; Basan et al., 2020; Okano et al., 2020; Gralka et al., 2023; Zhu and Dai, 2024).

### 7.1.2 Limits to microbial return on investment: physiological and molecular constraints

The return on investment depends on the intrinsic properties of the active microorganisms, the properties of the substrates that are available and the environmental conditions that can affect them. For instance, the modulation in physiological demand can occur through changes in the soil microbial community composition (Bahram et al., 2018; Lladó et al., 2018; Kaminsky et al., 2019) or alteration of their active metabolic pathways (Bergauer et al., 2018; Floudas, 2021; Gralka et al., 2023). These may result from eco-evolutionary processes, such as (i) the relative change in abundance of phylotypes within a microbial community, and (ii) mutations, changes in the allele frequency within a phylotype and horizontal gene transfers (Loreau et al., 2023; Abs et al., 2024). Secondly, the potential return on investment associated with microbial activity on a cellular level is constrained by the physicochemical properties of the reactants, such as the identity of the electron donors and acceptors, C-to-N ratio, redox state, concentrations, molecular weight, which can require costly homeostatic mechanisms or microbial strategies to overcome the bioenergetics of microbial reactions.

When properties of the substrates fall outside a microbial preferred range, metabolic flexibility needs homeostatic mechanisms to process the substrates. The phenomenon of homeostasis depicts the cellular processes that maintain metabolic fluxes, surface area-to-volume ratio (Kieft, 2000; Harris and Theriot, 2018; Belliveau et al., 2021), and elemental or redox stoichiometry (Manzoni et al., 2017; Mason-Jones et al., 2023; Moorhead et al., 2023; Flamholz et al., 2025), within a preferred interval through mechanisms of control and regulation, such as changing the concentrations of reactants and enzymes, and their kinetic and redox properties. Typically, homeostasis allows matching different compositions of organic substrates with one of the microbial biomass in terms of C chain size, nutrients (Heijnen and Kleerebezem, 2010; Ahamed et al., 2023) and redox state (e.g. more or less reduced or oxidized C) (Flamholz et al., 2025). Homeostasis is generally assumed in C and N cycling models, such as net N mineralization when there is N in excess and overflow respiration when there is C in excess because it is supported by empirical evidence of lower C use efficiency when C-to-N and C-to-P ratios are relatively higher than the one from biomass (Manzoni et al., 2017). However, redox homeostasis is not widely spread in models (Flamholz et al., 2025).

## 7.2 Microbial strategies to overcome bioenergetic constraints

### 7.2.1 Tuning the flow: strategies in a microbial world governed by kinetics and thermodynamics

Depending on the conditions, different members of a soil microbial community will be active. This will lead to different metabolic pathways being dominantly utilised, which in turn can have an effect on the kinetics and thermodynamics of the microbial reactions.

Under a given set of ecological constraints, a dominant proportion of the soil microbial community is in a dormant state, which is a state where the rates of microbial reactions and energy expenditure are generally reduced (Lennon and Jones, 2011; Leung et al., 2020). While microbial communities in bulk soil are generally partially active (Couradeau et al., 2019), a greater proportion of microorganisms is active in soil hotspots during short-term events or brief metabolic windows (Kuzyakov and Blagodatskaya, 2015; Leung et al., 2020; Li et al., 2024). Dynamic changes of reactant concentrations lead members of a microbial community to exhibit heterogeneous physiological states (Davey, 2011; Blagodatskaya and Kuzyakov, 2013; Bradley et al., 2018). Therefore, changes in the concentration of extracellular reactants constrain the fraction of the microbial community and the identity of their members that are activated (Schimel, 2018). This microbial activation would result in a modulation of active metabolic pathways (Wu et al., 2022; Zheng et al., 2023). This may be related to variation in the concentration of expressed transporters and enzymes, the intracellular composition of reactants, and the properties of enzymes, all of which have major impacts on the kinetics and thermodynamics of microbial reactions, or bioenergetics (Csete and Doyle, 2004).

The rates of biochemical reaction are mainly influenced by abiotic factors, such as soil moisture, soil temperature, soil structural properties, and chemical structure of reactants (LaRowe et al., 2014; Fanin et al., 2022; Moyano et al., 2013). Among the reactants, organic molecules can be involved in reactions where C-C bonds are formed or cleaved and redox reactions (Bar-Even et al., 2012a). Redox reactions are chemical transformations where electrons are transferred from a donor to an acceptor molecule. The study these redox reactions is important because their rates can be impacted by the diversity and composition of organic substances and catalysers, such as enzymes and microorganisms, factors that can be expected to be modulated by management, such as the diversification of agroecosystems.

Whatever the initial concentration of reactants, when a chemical reaction is at equilibrium, there is an absence of flux. At the equilibrium state, the ratio of substrate and product concentrations is characterised by a constant, the equilibrium constant. It results in the reaction rate being approximately related to the ratio of the concentrations of the products to the concentration of the substrates, namely the mass action ratio (West et al., 2024). The higher the activation energy of a reaction occurring in the absence of catalyst, the slower the rate of reaction. The equilibrium constant is mainly related to the temperature, pH and the reactants' chemical properties (Noor et al., 2012).

The chemical structures of reactants are generally used to calculate reduction potentials associated with a reaction. The notion of reduction potential explains the energetics of redox reactions and helps to predict whether a reaction is more or less energetically favourable (Bar-Even et al., 2012b). The reduction potential describes the tendency of functional groups in molecules to accept electrons. The following functional groups are increasingly oxidised and have a decreasing tendency to accept electrons: hydrocarbons, hydroxycarbon, carbonyls, and carboxyls. More oxidised C (with a higher nominal oxidation state of C or lower degree of reduction) in a functional group will tend to give electrons more readily and will be characterised by lower reduction potentials (Bar-Even et al., 2012b). In other words, for a given molecular species, the more negative the reduction potential, the lower the species' affinity for electrons and the lower the tendency to be reduced. The change in the reduction potential of a reaction (mV) is related to the change in Gibbs free energy (kJ /mol). The change in Gibbs free energy of a given reaction is a function of the pressure, temperature, pH, activities of reactants (that is related to the concentrations of reactants adjusted to the ionic strength of the solution, temperature and charges of molecular species) and the equilibrium constant (Amend and LaRowe, 2019). The gradient in reduction potential between electron donors and acceptors is generally related to redox reaction rates and the potential energy microorganisms can harvest (Plante et al., 2024; Zheng et al., 2024). This reduction potential gradient can be viewed as a driving force transferring electrons from donor to acceptor molecules from lower toward higher reduction potentials.

This driving force of *in vivo* biochemical reactions can, to a certain extent, be modulated by microorganisms, through changes in the kinetic properties and concentrations of enzymes and the concentrations of the substrates and products (Gerosa and Sauer, 2011; Davidi and Milo, 2017).

### 7.2.2 Against the flow: microbial strategies for difficult reactions

The reaction rate in active microorganisms is a function of at least two factors: the kinetic properties of enzymes and the thermodynamics associated with a reaction, such as how far the concentrations of reactants are from equilibrium (Davidi et al., 2018). A biochemical reaction associated with a higher activation energy may be viewed from a microbial perspective as physiologically more demanding because more cellular investment would be required to deal with this physicochemical constraint. However, even though enzymes allow the reduction of the activation energy of reactions, how physiologically demanding a reaction is does not generally correlate with kinetic properties of enzymes (Davidi et al., 2018). Yet, more demanding reactions associated with more difficult chemistry tend to require bigger architecture of the active catalytic sites (Arcus et al., 2016), which may represent an additional cost from a cellular perspective. In the case of biochemical reactions, catalysts or enzymes cannot modify the equilibrium concentrations of products and substrates (Davidi et al., 2018). One strategy to deal with reactions near equilibrium, which have unfavorable thermodynamics, is to increase the abundance of enzymes, and to a lesser extent, to alter the kinetic properties of enzymes (Davidi et al., 2018). Another strategy is to influence the mass action ratio (Noor et al., 2016).

The ratio between the product and substrate concentrations in physiological conditions, namely the mass-action ratio, can be dynamically modulated by microorganisms, due to the constrain of homeostasis, in response to changes in the nutritional status of the environment through:

(i) a homeostatic adjustment of their surface area-to-volume ratio (Harris and Theriot, 2016; Si et al., 2017), in addition to the expression of transporters, to modulate the efficiency for taking up substrates (Kieft, 2000; Bergkessel et al., 2016), which impact the cellular concentration of reactants, metabolic flux and *in fine* the generation time (Sjöstedt et al., 2012; Harris and Theriot, 2018);

(ii) the use of depolymerisation and solubilisation factors to promote the diffusion of substrates toward microorganisms (Schimel and Schaeffer, 2012; Manzoni et al., 2014; Sivadon et al., 2019; Li et al., 2021). Investing in extracellular enzymes or weathering compounds is suggested to influence the return on investment that soil microorganisms can potentially get (Schimel and Weintraub, 2003; Fontaine and Barot, 2005). One reason this may be relevant in soil systems is that processing limited amount of heterogeneous substrates supplied with poor nutritional values, may lead to bioenergetic constraints (Ekschmitt et al., 2005; Malik et al., 2019). These bioenergetic constraints was estimated using an index named potential energetic return on investment. This index was determined by dividing the energy content of organic substrates by their potential activation energy required to process them (Williams and Plante, 2018; Henneron et al., 2022);

(iii) the adaptation of the cytosolic volume occupancy (the extent to which cytoplasm solution is crowded) to influence the diffusion of reactants and catalytic efficiency of enzymes (Ellis, 2001; Minton, 2001; Beg et al., 2007; Pang et al., 2023).

Previously, I have indicated that microorganisms can influence the mass-action ratio and enzyme kinetics properties through various cellular strategies, such as the modulation of activities of reactants and properties of catalysers along metabolic pathways. However, they generally cannot modulate the equilibrium constant because it is related to the chemical structures of reactants. From the perspective of redox potentials, more “difficult” biochemical reactions, namely bottleneck biochemical reactions, can be viewed as reactions catalysed by enzymes where initially the flow of electrons can not spontaneously go from lower to higher physiological reduction potential unless these bottleneck reactions are activated (Bar-Even et al., 2012b). Alternatively, relatively more “difficult” biochemical reactions or chemistry may be viewed as very prolonged reactions (i.e. when their rate is relatively slower when not catalysed by enzymes in relation to their rate in enzyme-catalysed reactions) (Arcus et al., 2016). Due to the diversity of equilibrium constant and relative abundance of reactants, contrasting biochemical reactions can be characterised by varying chemistry complexity, such as the level of activation energy (Plante et al., 2024) or the availability of the enzymatic mechanism (Bar-Even et al., 2012a). Even though this is generally associated with a higher cellular energetic cost (Noor et al., 2014), microorganisms have strategies to ensure a net flux in the desired direction and to “free” themselves from these physicochemical constraints through:
(i) the coupling to energy-releasing transformations (i.e. ATP hydrolysis) for the activation of unfavourable reactions (i.e. carboxylation that is involved in the incorporation of inorganic C into organic molecules) (Bar-Even et al., 2012b; Jinich et al., 2018);
(ii) the use of alternative electron carriers than NAD(P)/NAD(P)$^+$ such as quinones or ferredoxins (Jinich et al., 2018; Floudas, 2021);
(iii) the modulation of the redox potential of enzymes (Ayala, 2010; Floudas, 2021; Taylor and Bhatnagar, 2024);
(iv) the variation of the size of the catalytic domain of enzymes (Arcus et al., 2016);
(v) the differential activation of metabolic pathways with a contrasting number of enzymatic steps which influence the proportion of bottleneck reaction and, *in fine*, the thermodynamic driving force (which results in a

trade-off between rates of reactions through the pathway and cofactors yield) (Bosatta and Ågren, 1999; Costa et al., 2006; Flamholz et al., 2013; Cueto-Rojas et al., 2015).

## 7.3 Influence of substrate diversities and composition on microbial resource allocation

Eventhough the pathway length may depend on the microorganisms' genetic makeup (Flamholz et al., 2013; Cueto-Rojas et al., 2015), it is also influenced by the composition of diverse substrates that is the focus of the thesis (Amon and Benner, 1994; Bosatta and Ågren, 1999; Gentile et al., 2024). Metabolic pathways that are characterised by a higher number of enzymatic steps may be activated by the presence of more complex molecules with a higher molecular weight, a higher number of C atoms and a lower C oxidation state (or higher energy content) (Bosatta and Ågren, 1999). Indeed, larger molecules or more reduced ones will generally result in a higher number of electrons transferred during the oxidation half-reaction of this compound (Ahamed et al., 2023). After a threshold, the number of electrons transferred may lead to some kinetic limitation in the electron transport chain (Heijnen and Kleerebezem, 2010; Zheng et al., 2024). This kinetic limitation may result in a reduced production rate of cofactors required to fuel the biosynthetic and polymerisation pathways, resulting in an overall lower microbial activity (Ahamed et al., 2023). Alternatively, the kinetic constraint that originates from the size of the molecules may be due to the proportion of colloids in the solution and their physicochemical properties (Gentile et al., 2024). However, these bioenergetics constraints that result from the physicochemical nature of the substrates taken up (Bosatta and Ågren, 1999; Costa et al., 2006; Flamholz et al., 2013; Cueto-Rojas et al., 2015) are thought to be relevant only in non-aerobic conditions, such as during fermentation (LaRowe and Amend, 2019; Endress et al., 2024). However, several exceptions exist suggesting that fermentation can co-occur with aerobic respiration at high microbial activity, and this can be related to the metabolic pathway lengths and topologies associated with bioenergetics constraints (Basan et al., 2015; Szenk et al., 2017; Niebel et al., 2019; Kreft et al., 2020). Ways to investigate the differential activation of these pathways have been developped (Herrmann and Bölscher, 2015; Robator et al., 2016, 2018; Chakrawal et al., 2020; Endress et al., 2024).

As previously stated, the traits of soil microbial community can be modulated by the change in agroecosystem management (Lepinay et al., 2024) impacting the amount and properties of organic inputs to soil, such as above- and below-ground litter and rhizodeposits (Hirsch et al., 2013; Kallenbach et al., 2015; Takriti et al., 2018; Martinović et al., 2022). However, it remains unknown what the actual chemical properties of both organic inputs and soil organic matter are (Stumpf et al., 2024). This prevents reliable prediction of soil microbial responses in terms of (i) the preferential processing of substrates (Weverka et al., 2023), (ii) the partition of metabolic flux (Wu et al., 2022) between small molecules and large molecules, such as gas, fermentation products, biomass, internal/external storage and external foraging capabilities (Geyer et al., 2020; Mason-Jones et al., 2023; Schnecker et al., 2023; Bölscher et al., 2024), and (iii) the magnitude and dynamics of microbial activity (Hungate et al., 2015; Singer et al., 2017; Wang et al., 2021a). The properties of diverse organic inputs, such as their molecular diversity, redox properties, energy content and chemical composition, may influence their bioavailability and biodegradability (Marschner and Kalbitz, 2003; Ahamed et al., 2023). Therefore, organic inputs with different properties are likely to constrain the temporal and overall activity of the soil microbial community that would face potential trade-offs, such as between resource acquisition, growth, yield, stress tolerance and survival (Zhu et al., 2022), while optimising the allocation of their cellular resources to maximise their potential return on investment (Basan et al., 2020; Okano et al., 2021).

## 7.4 Towards a bioenergetic and resource allocation framework for soil microbial communities

In the present thesis, it was shown that viewing microorganisms as resource-allocating self-replicators under the angles of redox chemistry (Flamholz et al., 2025), cellular resource allocation theory (Scott and Hwa, 2023) and kinetic theory of optimal pathway length (Kreft et al., 2020) is relevant for better understanding of soil microbial community responses to diverse inputs of organic substances with consequences for soil organic matter composition and dynamics. It was argued that an economical view of the functioning of soil microbial communities, with indicators such as the potential energetic return on investment that microbial communities may get through their cellular activities, may be a relevant, intellectually stimulating though challenging avenue for up-coming research in soil sciences. Even though, microorganisms and bioenergetics are very relevant aspects to investigate in soil systems (Naughton et al., 2021; Varsadiya et al., 2025; Wirsching et al., 2025; Yang et al., 2025), other factors may also be relevant to study and incorporate in models of organic matter dynamics (Craig et al., 2022), such as the soil structural heterogeneity from the perspective of the interaction between soil inorganic and organic chemistry (Kleber et al., 2021), soil physics (Nunan et al., 2017; Meurer et al., 2020) and the food web ecology (Erktan et al., 2020; von Bommel et al., 2024). Questions about the functioning of soil microbial community remain (Blagodatskaya and Kuzyakov, 2013; Singer et al., 2017; Wu et al., 2022; Shao et al., 2024). For instance, when not only individual substrates (Geyer et al., 2020) but mixtures are used as inputs to soils, such as small molecules in combination with polymers and/or chains and fractal colloidal clusters (Algora et al., 2022; Gentile et al., 2024), what are the resulting *in situ* microbial activity, C, nutrients and energy partitions into different metabolic pathways and products and the consequences on soil health?

From this appendix section and the conclusions from the present thesis, I would like to propose the following two hypotheses that would remain to be tested in contrasting soil systems:

**(i)** Good quality of bioavailable mixed C substrates, which are a more concentrated energy source characterised by a high return on investment when processed, will result in a higher microbial growth rate or higher growth efficiency (Roller and Schmidt, 2015; Kästner et al., 2021) and control an allocation toward more proteins involved in biosynthesis of buildings blocks, more ribosomes (Scott and Hwa, 2023) and more internal/external storage (Mason-Jones et al., 2022). This would lead to a more oxidised microbial biomass (Flamholz et al., 2025). I also could expect a more crowded cytoplasm (Pang et al., 2023) and a less crowded envelope (Scott and Hwa, 2023), a lower investment in motility (Ni et al., 2020), a lower surface area-to-volume ratio (Bergkessel et al., 2016) and peripheral metabolic pathways with a lower length (Wang and Kuzyakov, 2024). A higher rate of microbial necromass (Wattenburger et al., 2023) and by-products formation that, *in fine*, stimulate soil organic C accumulation with a microbial origin (Perveen et al., 2019; Wang et al., 2021b) can be assumed.

**(ii)** Mixed bioavailable C compounds of bad quality, more diffuse energy source of low return on investment will result in a lower microbial growth rate or lower growth yield and would lead to a proteome allocation toward more proteins involved in peripheral pathways and fewer ribosomes and proteins involved in biosynthesis and depolymerisation due to the protein density constraint. This would lead to a more reduced microbial biomass (Flamholz et al., 2025). I also expect a higher investment in motility, a more crowded envelope, a less crowded cytoplasm, a higher surface area-to-volume ratio and peripheral pathways with a higher length. In this context, I could expect a lower rate of microbial necromass. In particular, the increase of secretion of "solubilisation factors" (e.g. extracellular enzymes and organic acids) due to the supply of bad quality substrate to soil microorganisms which could "weather" soil organic C content (Fontaine et al., 2007; Jiling et al., 2021; Li et al., 2021).

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# Popular science summary

## What soils can teach us about diversity, diets, and energy: a beautiful journey through the dirt beneath our roots.

Soil is teeming with life! Soil microorganisms, those organic creatures that are invisible to our eyes, thrive in a mineral world. This labyrinth of pores filled with water, air and organic matter, molecules partly made of carbon, hydrogen, oxygen atoms, is their home. As the biological engine of the Earth, microbes drive many of the key services which soils deliver. Similar to us, they require energy to thrive, and they acquire it from the decomposition of organic matter, the ‘food’ needed for their survival.

Diversity helps human societies to thrive. A world with only one language, one ideology, one diet would be rather unpleasant, wouldn’t it? Just as our health benefits from a varied meal, we might expect that the health of soils, home to unknown and fantastic microbes yet to be discovered and studied, would also benefit from a more diverse 'flexiterian diet' of plant and faunal material. After all, when we manage the land by planting crops, grasses, shrubs and trees, we organise a menu of leaves, roots and their sweat that ends up in the soil. But does this diversity actually help the microorganisms to be more active?

This thesis asked that very question: Does giving soil microbes a more diverse buffet affect how they break down molecules, grow and set up their home? The short answer? It depends.

### Paper I – Energy matters more than diversity

To understand how soil microorganisms respond to different inputs, I looked not just at *what* they were being fed, but at *how energetically rewarding* that food was. Think of it this way: a bowl of dumplings with rice might give you more energy than a hot soup with fish and sour vegetables. Similarly, microbes respond more to **how much energy they can get back** from breaking down organic matter than to how diverse it is.

Using tools like bomb calorimetry (a way of measuring the energy in food) and mass spectrometry (a way to measure whether or not what you have eaten has poisoned you), I discovered that soils that offer a more attractive menu to their inhabitants, with a more 'accessible' energy, like those in grasslands, support more active microorganisms than those in woodlands. It was not the variety of food on the buffet that mattered, but the amount of **usable** energy in the dish. So in economic terms, the microorganisms were looking for a better **return on investment**, more energy back for the effort they put in to consume acquire and consume the food.

### Paper II – Microorganisms in soil are picky (but not always)

I then tested what happens when microbes are offered cocktails of exudates, the sweat of plant roots. Interestingly, I found that microorganisms have **preferences** and usually pick and transform their favourite substrate first, usually the molecules that allow them to be the most active. However, when there were only a few options available to them on the menu, the microorganisms ate their food in a more interactive way.

### Paper III – The cousin rather than the sibling plant above modifies the diversity of the soil pantry below

I finally looked at how different willow (*Salix*) plants grown over time changed the chemical makeup of the organic matter in the soil. While differences between willow species, but not varieties, led to subtle changes in the diversity and composition of organic molecules in the soil, the effects were small and sometimes hard to see because soils naturally vary a lot. I also compared a cheap with an expensive technique to study the diversity and composition of the soil pantry using both mid-infrared spectroscopy and mass spectrometry coupled with a prior step where the large organic molecules are warm-up and broken down into smaller molecules. The two approaches made it possible to distinguish the impact of the willow on the soil chemical makeup, but not in the same way. This suggests that more work needs to be done if the expensive instrument is to be replaced by the cheaper one.

### So, what does it all mean?

The key message from my thesis is that **services delivered by soil related to organic matter dynamics and microbial activity aren't just about having more diverse plant inputs**, but about the **chemical and energetic quality** of those inputs. Microorganisms, like us, care about the effort it takes to get their energy. If we want healthier soils, and carbon staying longer in soil instead of going to the atmosphere (which help mitigate climate change), we should focus on **what kind of energy we are putting into soil**, not just how varied it is.

So, while plant diversity remains valuable for agroecosystems, when it comes to microbial communities in soils, it is less about how diverse the menu is and more about how **fuel-efficient** their meals are.

# Populärvetenskaplig sammanfattning

**Vad jordar kan lära oss om mångfald, dieter och energi: en vacker resa genom jorden under våra rötter.**

Jorden kryllar av liv! Markmikroorganismer, de organiska varelser som är osynliga för våra ögon, frodas i en värld av mineraler. Denna labyrint av porer fyllda med vatten, luft och organiskt material (molekyler som delvis är uppbyggda av kol-, väte- och syreatomer) är deras hem. Som jordens biologiska motor driver mikrober många av de nyckelprocesser och funktioner som jordar levererar. I likhet med oss kräver de energi för sina dagliga liv, och det får de genom att bryta ner organiskt material, den "mat" som behövs för att de ska överlevnad.

Mångfald hjälper mänskliga samhällen att frodas. En värld med bara ett språk, en ideologi, en diet skulle vara tråkig, eller hur? Precis som vår hälsa gynnas av att äta varierat kan man tänka sig att marken, hem för okända och fantastiska mikrober som ännu inte har upptäckts och studerats, också skulle gynnas av en mer varierad kost av växt- och djurmaterial. När vi sköter marken genom att plantera grödor, gräs, buskar och träd, erbjuder vi en meny av löv, rötter som hamnar i jorden. Men hjälper denna mångfald verkligen mikroorganismerna att bli mer aktiva?

Den här avhandlingen ställde just den frågan: Påverkar en mer mångsidig buffé hur markmikrober växer och bryter ner molekyler? Det korta svaret är att det beror på.

## Paper I – Energi betyder mer än mångfald

För att förstå hur markmikroorganismer reagerar på att erbjudas en variation av mat tittade jag inte bara på vad det var för typ av mat, utan också på hur energirik maten var. Tänk på det så här: en skål med dumplings och ris kan ge dig mer energi än en varm soppa med fisk och grönsaker. På liknande sätt reagerar mikrober mer på hur mycket energi de kan få genom att bryta ner organiskt material än på hur varierat det är.

Med hjälp av verktyg som ”bomb kalorimetri” (ett sätt att mäta energin i maten) och masspektrometri (ett sätt att mäta om det du har ätit har förgiftat dig eller inte), upptäckte jag att jordar som erbjuder en mer attraktiv meny för sina invånare, med mer "tillgänglig" energi, som gräsmarker, stödjer mer aktiva mikroorganismer än skogsmarker. Det var inte variationen på maten på buffén som spela roll utan mängden användbar energi. Så i ekonomiska termer letade mikroorganismerna efter en bättre avkastning på investeringen, mer energi tillbaka för den ansträngning de lagt ner.

## Papper II – Mikroorganismer i marken är kräsna (men inte alltid)

Jag testade sedan vad som händer när mikrober erbjuds cocktails av rotexudat, växtrötternas svett. Intressant nog upptäckte jag att mikroorganismer har preferenser och vanligtvis väljer och transformerar sitt favoritsubstrat först, vanligtvis de molekyler som gör det möjligt för dem att vara mest aktiva. Men när det bara fanns ett fåtal alternativ tillgängliga för dem på menyn åt mikroorganismerna sin mat på ett mer interaktivt sätt.

## Papper III – Kusiner snarare än syskonväxter ovanjord ändrar mångfalden i skafferiet i marken

Jag tittade slutligen på hur olika pilväxter (*Salix*) som odlades över lång tid förändrade den kemiska sammansättningen av det organiska materialet i marken. Medan skillnader mellan pilarter, snarare än sorter, ledde till subtila förändringar i mångfalden och sammansättningen av organiska materialet i marken, var effekterna små och ibland svåra att se eftersom jordar varierar mycket naturligt. Jag jämförde också en billig och en dyr teknik för att studera sammansättningen av det organiska materialet, infraröd spektroskopi och masspektrometri i kombination med ett tidigare steg där de stora organiska molekylerna värms upp och bryts upp i mindre molekyler. De två metoderna gjorde det möjligt att särskilja pilens inverkan på den kemiska sammansättningen av det organiska materialet i marken, men inte på samma sätt. Detta tyder på att mer arbete måste göras om den dyra metoden ska ersättas med det billigare.

## Så, vad betyder resultaten?

Huvudbudskapet från min avhandling är att tjänster som levereras av marken relaterade till organiskt material och mikrobiell aktivitet inte bara handlar om att ha stor variation i växtmaterial som tillförs, utan om den kemiska kvaliteten och energiinnehållet i växtmaterialet. Mikroorganismer, som vi, bryr sig om den ansträngning som krävs för att få sin energi. Om vi vill ha friskare jordar och att kol stannar längre i marken istället för att försvinna till atmosfären (vilket hjälper till att mildra klimatförändringarna), bör vi fokusera på vilken typ av energi vi tillför marken, inte bara hur varierad den är.

Så även om mångfalden av växter är värdefull för många aspekter i agroekosystem, handlar det mindre om hur varierad menyn är och mer om hur bränsleeffektiva måltiderna är, när det gäller det mikrobiella samhället i marken.

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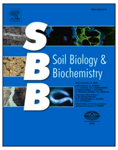

# Potential energetic return on investment positively correlated with overall soil microbial activity

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## ABSTRACT

Microbial communities are a critical component of the soil carbon (C) cycle as they are responsible for the decomposition of both organic inputs from plants and of soil organic C. However, there is still no consensus about how to explicitly represent their role in terrestrial C cycling. The objective of the study was to determine how the molecular and energetic properties of readily available organic matter affect the metabolic activity of the resident microbial communities in soils. This was achieved by cross-amending six soils, taken from woodland and grassland sites along an urban pressure gradient, with organic matter extracted from the same six soils and measuring heat dissipated due to the increase in microbial metabolic activity. The energetic properties of the organic matter were used to estimate a potential energetic return on investment (ROI) that microbial communities could obtain from the transformation of the organic matter. Specifically, the ROI was calculated as the ratio between the total net energy available ($\Delta E$) and the weighted average standard state Gibbs energies of oxidation half reactions of organic C ($\Delta G^{\circ}_{Cox}$). $\Delta E$ was measured as the heat of combustion using bomb calorimetry. $\Delta G^{\circ}_{Cox}$ was estimated using the average nominal oxidation state of C (NOSC) of the molecular species in the organic matter. The overall metabolic activity of microbial communities was positively related to the potential energetic return on investment but no significant relationship was found with the molecular diversity of organic matter. The temporal differences in metabolism across soils indicate that bacterial communities do not exploit the potential energetic return on investment in the same way: the suburban grassland communities responded more rapidly and the suburban woodland communities more slowly to the organic matter additions than the other communities. The urban gradient did not affect the properties of the molecular or energetic properties of the organic matter nor the response of the microbial communities to the organic matter additions. However, the organic matter from the grassland soils caused soils to dissipate 36.4% more heat than organic matter from the woodland soils. The metabolic response was also more rapid after the addition of grassland organic matter: the time taken for half the heat to be dissipated was 6.4 h after the addition of grassland organic matter and 6.1 h after the addition of woodland organic matter. Overall, our results suggest that microbial communities preferentially use organic matter with a high potential energetic return on investment, i.e. organic molecules that do not require high cost associated with catalysis whilst yielding a high net energetic benefit.

## 1. Introduction

The mineralisation of soil organic C by microbial decomposers releases approximately 6 times the amount of $CO_2$ to the atmosphere than do anthropogenic emissions (Ballantyne et al., 2017). Therefore, even small changes in this flux can have significant effects on future

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*Abbreviations:* ROI, Energetic return on investment; $\Delta E$, Heat of combustion; NOSC, Nominal oxidation state of carbon; $\Delta G^{\circ}_{Cox}$, Standard state Gibbs energies of oxidation half reactions of organic carbon.

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atmospheric $CO_2$ levels. There is a general consensus that microbial access to substrate, organic matter association with mineral surfaces and anaerobic conditions all constrain microbial decomposition of soil organic matter and contribute to organic C persistence in soil (von Lützow et al., 2006; Dignac et al., 2017; Keiluweit et al., 2017). The hierarchy of involvement of these mechanisms in regulating organic C persistence is also believed to change as a function of soil physico-chemical properties (Rasmussen et al., 2018). However, whilst these mechanisms explain why organic C remains in soil over the longer term, they are less useful for understanding decomposition rates in microbial activity hotspots, which have been estimated to account for the majority of $CO_2$ emissions from soils (Kuzyakov and Blagodatskaya, 2015).

Microbial hotspots are sites with significantly higher microbial activity than the surrounding bulk soil, examples of which are the rhizosphere or the detritusphere (Kuzyakov and Blagodatskaya, 2015). They differ from the bulk soil in that there is a non-limiting supply of organic substrate for the period of existence of the hotspot (Kuzyakov and Blagodatskaya, 2015). When there is ample supply of substrate, the abiotic constraints on microbial activity are negligible and the decomposition of organic C is more likely to be related to the intrinsic properties of the microbial decomposers and the properties of the available organic matter. Carbon processing by microbial communities depends on the type of microbial metabolism and the energetic requirements of the cellular processes present (LaRowe and Amend, 2015; Smeaton and Van Cappellen, 2018). This affects both $CO_2$ emissions and the production of different forms of organic C, which might result in different levels of C persistence in soil (Sokol and Bradford, 2019). Recent evidence also suggests that the taxonomic composition and diversity of the microbial communities affect how substrate C is processed in soil (Saifuddin et al., 2019; Domeignoz-Horta et al., 2020). For example, the energetic requirements for synthesizing cellular biomass can vary by up to four orders of magnitude, depending on cell size and environmental conditions (LaRowe and Amend, 2016). Therefore, there may be thermodynamic constraints on microbial metabolic activity.

The nature of the organic C being consumed also has some bearing on how, and the rates at which, it is processed. It has generally been assumed that the thermodynamic properties of substrate (i.e. the energy required to activate the oxidation of electron donors) do not influence microbial respiration under aerobic conditions because the availability of oxygen as a terminal electron acceptor means that sufficient energy is produced for ATP generation. However, in sediments it has been shown that microbial respiration can indeed be related to the thermodynamic properties of organic matter under certain conditions (Garayburu-Caruso et al., 2020). In soils, it has long been established that organic substrates stimulate microbial activity to a greater or lesser extent, depending on their nature (Enwall et al., 2007) and concentration (German et al., 2011). The microbial carbon use efficiency (i.e. the amount of microbial biomass-C produced per unit organic C consumed) is similarly related to the molecular nature of the substrate (Bölscher et al., 2017; Jones et al., 2018). However, the organic matter that is available to decomposers in soil displays a high degree of molecular heterogeneity (Swenson et al., 2015) and the effects of heterogeneous substrate on microbial activity is, as yet, unclear (Jones et al., 2018). Microorganisms acquire resources from outside the cell and, in doing so, incur an energetic cost associated with the production of extracellular enzymes and membrane transport proteins (Nunan et al., 2020). There is, therefore, a greater metabolic cost associated with the acquisition of heterogeneous resources due to the necessary production of a broader range of enzymes and uptake apparatuses, which would be expected to lower the cellular biomass yield and increase $CO_2$ emissions (Allison et al., 2014). However, individual microorganisms have a limited substrate range (Nunan et al., 2020) and increases in substrate heterogeneity are therefore likely to lead to increases in the diversity of community members consuming the substrates. The metabolic cost is shared by a greater proportion of the community and the cost incurred by individual microorganisms does not necessarily increase.

The ultimate outcome of microbial processing of heterogeneous organic substrate depends upon the return on investment that microbial decomposers obtain when acquiring resources in soil (Lehmann et al., 2020). The idea that the return on investment plays a significant role in the dynamics of C in soil has been proposed on repeated occasions in the literature (Schimel and Weintraub, 2003; Rovira et al., 2008; Allison et al., 2010; Barré et al., 2016; Wutzler et al., 2017; Williams and Plante, 2018). Whilst this is conceptually appealing, there is no empirical evidence of energetic return on investment being related to the microbial decomposition of organic substrate in soils. The energetic return on investment can be defined as the efficiency of energetic investments and can be calculated by dividing the net energetic benefit by the direct cost of metabolic pathways involved in the transformation of organic substrates by microorganisms. Therefore, it should be possible to estimate the energetic return on investment using empirical thermodynamic, kinetic and physiological data in metabolic network models (Jin and Bethke, 2003; Niebel et al., 2019). However, the empirical data required to parametrise such models are not available for the large diversity of organic compounds (Noor et al., 2012), enzymes (Davidi and Milo, 2017) and microorganisms found in soil (Cavalier-Smith, 2010; Henry et al., 2016). This explains why the empirical evidence about the dynamics of C response to soil microbial energetics has been rather thin on the ground.

Heterotrophic microbial cells derive energy to produce ATP from the oxidation of organic matter. Soil organic matter containing carbon atoms that are more reduced on average tend to require a higher energy for their electrons to be removed and their carbon-carbon bonds to be cleaved (Weber, 2002; Bar-Even et al., 2012a, b; Jinich et al., 2018). They have higher Gibbs free energy for the oxidation half reactions of organic carbon, on a C-mole basis ($\Delta G^{\circ}_{Cox}$) (LaRowe and Van Cappellen, 2011). The $\Delta G^{\circ}_{Cox}$ therefore indicates an approximation of the actual energy that microbial communities must invest in order to oxidize the organic matter.

Based on this rational, we propose an experimental approach to estimate a potential energetic return on investment that microbial decomposers can acquire from the transformation of organic matter. The metric is determined as the ratio between the total energy available in the organic substrate ($\Delta E$) and the $\Delta G^{\circ}_{Cox}$ of the molecular species contained in the organic matter. We estimated the $\Delta G^{\circ}_{Cox}$ using the nominal oxidation state of carbon (NOSC), which was deduced from the elemental composition of the molecular species (LaRowe and Van Cappellen, 2011; Willems et al., 2013). We determined the $\Delta E$ by bomb calorimetry (Harvey et al., 2016).

The objective of the study was to determine how the potential return on investment available to microbial communities is related to their metabolic activity in response to added organic matter. We also aimed to determine how properties of the organic matter (composition, molecular or energetic heterogeneity) and of the resident soil microbial communities (community structure) affect this relationship. In order to achieve this we used six soils, taken from woodland and grassland sites along an urban pressure gradient. The soluble organic matter of woodland soils is known to contain larger molecules than that arable or grassland soils, whereas grassland soluble organic matter contains more smaller molecules, such as amino acids and carbohydrates (Chantigny, 2003). Furthermore, urban pressure has been shown to affect microbial decomposition of organic matter, with labile organic material being decomposed more rapidly and more recalcitrant material more slowly in urban environments (Kotze and Setälä, 2022). This difference may be due to the fact that urban management practices select for copiotrophic organisms (Thompson and Kao-Kniffin, 2019).

Our hypotheses were that: i) the organic matter from the woodland soils is less decomposed than the organic matter from the grassland soils because it is composed of larger molecules that require a greater investment from microbial decomposers; ii) the urban pressure gradient is positively related to microbial processing of the organic matter due to more copiotrophic microbial communities; iii) the molecular

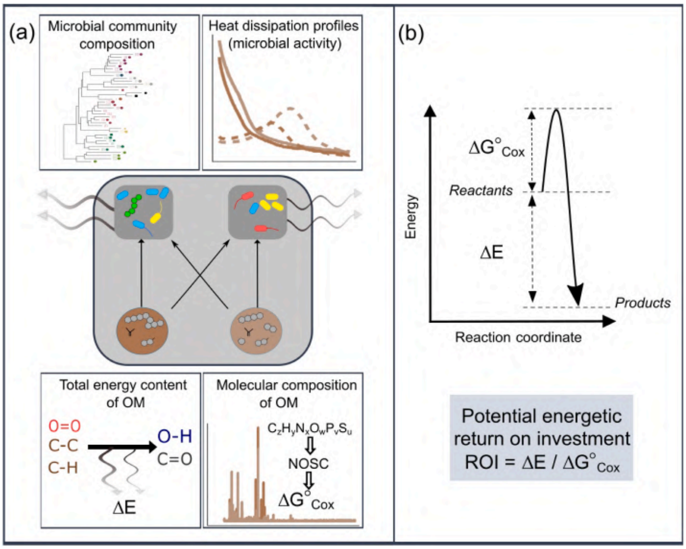

**Fig. 1.** Conceptual representation of the experimental design. (a) Six soil organic matter solutions containing different molecular profiles but the same quantity of organic C were added cross-wise to 6 soils (central panel). The soils harboured distinct bacterial communities (top left panel). The metabolic responses of microbial communities to the addition of soluble organic matter were determined as heat dissipation (wavy arrows) dynamics during a 24 h period (top right panel). The total energy content (ΔE) was measured by bomb calorimetry (bottom left panel) and $\Delta G^{\circ}_{Cox}$ was estimated from the molecular composition of substrates determined using Fourier transform ion cyclotron resonance mass spectrometry (bottom right panel). **(b)** The potential energetic return on investment (ROI) was calculated as the ratio between ΔE and the weighted average $\Delta G^{\circ}_{Cox}$.

heterogeneity of the added organic matter is negatively related to microbial activity, due to the higher cost involved in metabolizing more heterogeneous organic matter; iv) the greater the estimated potential energetic return on investment available to microbial decomposers in organic matter, the greater the metabolic activity.

## 2. Materials and methods

### 2.1. Experimental design

Our experiment was achieved by cross-amending six soils with excess soluble organic matter extracted from the same six soils and measuring the heat dissipated due to the increase in microbial metabolic activity (Fig. 1). The excess organic matter was added in order to create conditions that were similar to what might be found in activity hotspots. The composition of the organic matter amendments was determined using ultra high resolution mass spectrometry and the elemental composition of each molecular species in the organic matter amendments was used to estimate the nominal oxidation state of carbon (NOSC) of the molecular species, from which the $\Delta G^{\circ}_{Cox}$ were calculated (LaRowe and Van Cappellen, 2011). The organic matter heterogeneity was estimated as the diversity of molecular compounds and as the diversity of NOSC present in the organic matter. In order to determine how different microbial communities affect the relationship between energetic return on investment and microbial activity, we also measured the taxonomic composition of the communities in each of the six soils (Fig. 1).

### 2.2. Soil and soluble organic matter

Soils were sampled in June 2016 from six sites (Table S1), associated with two land-use types (woodland or grassland), along an urban pressure gradient (rural, suburban and urban areas) (Foti et al., 2017). Here, woodland is used to describe wooded areas and grassland is used to describe public parks with grass cover. These soils were chosen based on the results from a previous study (Foti et al., 2017) on the same sites where variations in soil texture, pH and total phosphorus content were observed (Table S1). Therefore, we expected them to harbor a range of soluble organic matter compositions and microbial communities. Three subsamples were taken from the surface 10 cm after removal of the litter layer. The inter-subsample distance was at least 5 m. The soil was then sieved (< 2 mm), mixed and one portion was stored at 4 °C until water soluble OM was extracted (within two weeks of sampling) and freeze-dried. The freeze-dried material was then analysed for total C and N. The remainder of the freeze dried material was stored in sealed, dark containers until analysis by ultra high resolution mass spectrometry/bomb calorimetry or re-solubilised and used to amend the soils in the isothermal calorimetric experiment (see below). Another portion of soil was stored at −20 °C for up to 10 weeks. The frozen soil was used for characterizing the bacterial communities and the isothermal calorimetric experiment (see below).

Water-soluble organic matter was extracted in triplicate by shaking soil samples with $H_2OmQ$ (1:10 soil:water) at 60 °C for 30 min and subsequently centrifuging the soil suspension (5250×*g*) for 10 min at 4 °C (Nkhili et al., 2012). The supernatant was filtered through glass fiber filters (pore size 0.7 μm, Sartorius). The filtrate was freeze-dried and the resulting material was stored at room temperature in the dark. The total organic C and total N content of the soluble organic matter and of the soils were determined using an elemental analyser that had been calibrated with tyrosine (Tables S1 and S2). Prior to analysis, the inorganic carbon of the soluble organic matter was removed by acid fumigation (Harris et al., 2001).

### 2.3. Molecular and energetic analysis of soluble organic matter

The total energy available in the soluble organic matter was measured as the heat of combustion with bomb calorimetry (Harvey et al., 2016). The instrument was a Parr Oxygen Bomb Calorimeter 6300 M20609 (Parr instruments Moline, Illinois, USA). Calibration samples were always measured first with Benzoic Acid standardized for bomb calorimetry (Parr no. 3415, CAS.reg 65-85-0). Measurements were not replicated because the maximum variation that has been previously observed when duplicates were analysed was found to be 1.5% (data not shown). Values for heat of combustion (ΔE) were converted into J $mmol^{-1}$ C. There was insufficient sample to reliably measure the ΔE for the soluble organic matter of rural woodland soil.

The molecular composition and diversity of the soluble organic matter was measured by ultra high resolution mass spectrometry prior to the heat dissipation experiment. These data were then used to derive the NOSC of the molecular species contained in the different soluble organic matter (LaRowe and Van Cappellen, 2011).

Ultra high resolution electrospray ionization Fourier-transform ion cyclotron resonance (ESI FT-ICR) mass spectra were acquired on a Bruker SolariX XR hybrid quadrupole-ICR mass spectrometer (Bruker Daltonics, Bremen, Germany). ESI FT-ICR is equipped with Paracell™ dynamic harmonization, an actively shielded 7 T superconducting magnet and an electrospray ionization (ESI) source (Bruker). Freeze-dried soluble organic matter was first solubilised in 25% MeOH, and 75% high quality grade and ultrapure water in order to prevent reaction of compounds in solution (McIntyre and McRae, 2005). The samples were diluted 30 fold in water/methanol (50/50 v:v) and infused continuously at a flow rate of 2 μl $min^{-1}$ in positive ionization mode at 4 kV. Nitrogen was used both as drying gas at a flow rate of 4 l $min^{-1}$ and nebulizing gas at a pressure of 1 bar. The temperature of the source was kept at 200 °C. Mass spectra were recorded over a mass range of m/z 50–1000 targeting a resolution of 0.5–2M according to m/z. External calibration was always performed prior to sample analysis using the G24221A Tuning Mix calibration standard from Agilent Technologies (Santa Clara, CA). This was done by setting a signal-to-noise ratio equal to 3, reaching accuracy values lower or equal to 700 ppb. The spectra were acquired with a time domain of 16 megawords and twenty scans for fifty ms were accumulated for each mass spectrum. A control sample containing only the solvent mixture (water/methanol (50/50 v:v) was systematically analysed and the resulting spectrum was subtracted from the spectra of the subsequent sample analysed. Data processing was done using Compass Data Analysis 4.1 (Bruker).

The assignment of molecular formulae from the detected mass-to-charge ratio (m/z) was performed using the TRFu algorithms (Fu et al., 2020) (version: TRFuFTMSopen07122020). The following formula assigning parameters were employed: the maximum mass error ($\Delta mc = 1010$ ppb), $0.3 = H/C \leq 2.5$, $0 < O/C \leq 1.25$, $4 = C \leq 50$, $0 \leq 13C \leq 1$, $N \leq 5$, $P \leq 1$, $S \leq 3$, singly charged ions in positive mode (max_charge = 1), - 0.5 < double bond equivalent (min_DBE), the maximum intensity derivation of $^{13}C$ isotopic peak compared with the theoretical value is 30% (tol_br = 30), no execution of the DBE-O rule (AquaDOM = 0). The resulting neutral molecular formulae were classified into biochemical categories using a multidimentional stoichiometric compound classification approach (Rivas-Ubach et al., 2018).

The NOSC was calculated from the neutral molecular formula estimated from each mass-to-charge ratio detected according to LaRowe and Van Cappellen (2011).

$$\text{NOSC} = 4 - [(4C + H - 3N - 2O + 5P - 2S) / C] \qquad (1)$$

where C, H, N, O, P and S refer to the stoichiometric number of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur atoms per molecular formula. This equation assumes the oxidation states of the atoms (C = + 4, H = + 1, N = - 3, O = - 2, N = - 3, P = + 5 and S = - 2) and the neutrality of organic molecules.

Based on the molecular composition and diversity of the soluble organic matter, we deduced the composition and diversity of the NOSC from the elemental composition of the molecular species present in the soluble organic matter (LaRowe and Van Cappellen, 2011). Diversity indices of soluble organic matter were estimated using the richness and the effective Simpon index of the molecular formulae and NOSC (Jost, 2007; Lagkouvardos et al., 2017).

The sum of the intensity weighted NOSC of each soluble organic matter was calculated as follows:

$$\text{Sum of the intensity weighted NOSC} = \Sigma\,(\text{NOSC} \times \text{RI}_{\text{NOSC}}) \qquad (2)$$

where $RI_{NOSC}$ is the relative intensity of each NOSC in the mass spectra. It has been shown that the NOSC is correlated with the standard state Gibbs energies of oxidation half reactions of organic compounds ($\Delta G°_{Cox}$) (LaRowe and Van Cappellen, 2011). As the $\Delta G°_{Cox}$ of each molecular formula is additive, the bulk $\Delta G°_{Cox}$ of each soluble organic matter was calculated in J $mmol^{-1}$ of C at 25 °C, 100 kPa as follows:

$$\Delta G°_{\text{Cox}} = 60.3–28.5 \times \text{Sum of the intensity weighted NOSC} \qquad (3)$$

The energetic return on investment (ROI) that microbial decomposers can potentially extract in aerobic condition during the transformation of the soluble organic matter was calculated as follows:

$$\text{ROI} = \Delta E \;/\; \Delta G°_{\text{Cox}} \qquad (4)$$

where ΔE is the total net energy available (determined by bomb calorimetry) and $\Delta G°_{Cox}$ is the standard state Gibbs energy of oxidation half reaction of organic C (determined using ESI FT-ICR-MS); both entities are in J $mmol^{-1}$ of C. We assume that $\Delta G°_{Cox}$ is proportional to the change in Gibbs energy associated with the oxidation of organic molecules in non standard conditions (where the actual activities of all reactants, the pH and the ionic strength in soils that have received the different soluble organic matter are taken into account) (Amend and LaRowe, 2019).

### 2.4. Soil microbial metabolic response to additions of soluble organic C

In order to determine the metabolic response of different microbial communities to a range of heterogeneous organic matter, we cross amended the six soils with re-solubilised organic matter from each of the soils and measured microbial metabolic activity by isothermal calorimetry for 24h (Fig. 1). All treatment combinations were carried out in quadruplicate (n = 6 soils × 7 treatments × 4 replicates = 168 samples) and they were analysed in a random sequence. Prior to the calorimetric experiment the soils were incubated for 4 days at 25 °C and at a matric potential of −0.033 MPa in order to standardise the conditions in the soils. The experiment was setup by placing aliquots of soil (5 g dry weight equivalent) into 22 ml glass reaction vessels. The organic matter solutions (0.1 ml; 0.3 mg $C_{org}$ $g^{-1}$ soil dry weight) or $H_2OmQ$ (control condition) were then added drop-wise. The reaction vessels were sealed with a lid (acid proof stainless steel with O-ring seal) and set carefully inside a TAM Air isothermal calorimeter (TA Instruments Sollentuna, Sweden) with a thermostat set to 25 °C. Heat dissipation (μW $g^{-1}$ soil dry weight) was measured continuously for 24 h. Heat dissipation data was chosen as a measurement of the microbial metabolic response because it gives a more complete and robust measurement of microbial activity than do $CO_2$ emissions (Herrmann et al., 2014). Heat dissipation measurements during the first hour were discarded as the signal was affected by the disturbance of the experimental setup. The heat dissipation due to microbial metabolism of the added organic matter was determined by subtracting the heat dissipation in the $H_2OmQ$ treatment.

### 2.5. Soil bacterial community analysis

The bacterial community structure was analysed after extraction of soil DNA, amplification and sequencing of the V3–V4 region encoding for the 16S rRNA sequences. Prior to the heat dissipation experiment, the initial bacterial communities in the six soil samples were analysed in triplicate. However, the sequencing quality was insufficient to reliably measure the bacterial community composition for one of the replicates of the suburban grassland soil.

Total DNA was extracted from the 0.5 g soil samples (wet weight) of each site with a FastPrep-24 bead beating system (MP Biomedicals, Solon, OH, USA) in combination with a FastDNA Spin kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. Total DNA was purified by elution through a GeneClean Turbo column (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. Concentration of the resulting cleaned DNA was determined

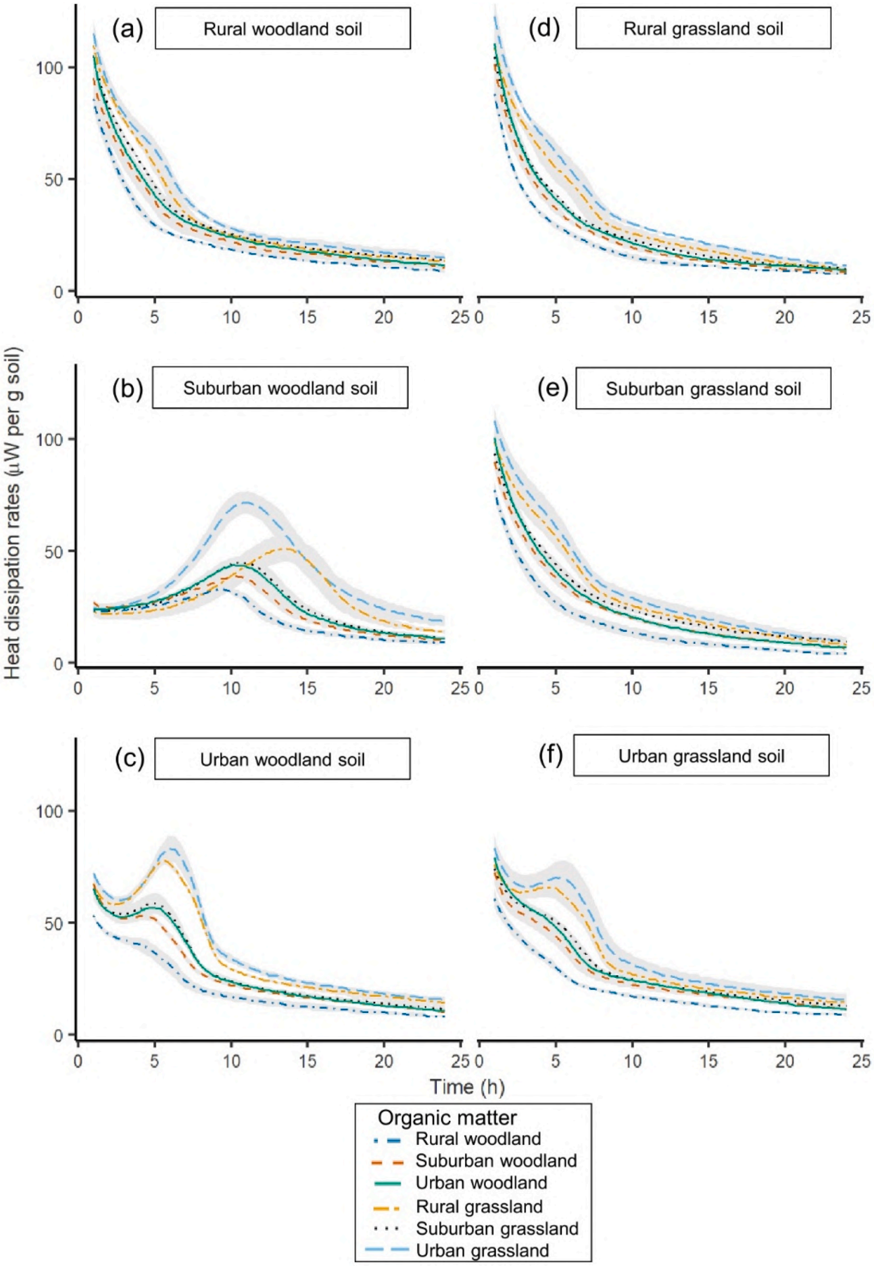

**Fig. 2.** Heat dissipation patterns from soils after the addition of 0.3 mg soil organic carbon. **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soils. Each curve depicts the mean (n = 4) heat dissipation after subtraction of the mean heat dissipation in control soils that only received water. The grey envelopes around the curves are the standard deviations.

using a fluorometer (Qubit®*ds*DNA HS) (data not shown).

The sequencing was carried out by MrDNA-Molecular Research (www.mrdnalab.com, Shallowater, TX, USA) as follows. First, the V3–V4 variable region encoding for the 16S rRNA sequences was amplified using the primers 341F-785R (with barcode on the forward primer), using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. After library preparation, Illumina Miseq sequencing (2*250 bp) was performed following the manufacturer's guidelines. The raw sequence data were processed using MR DNA analysis pipeline. In summary, reads were merged and barcodes were removed after trimming. Sequences <150bp and sequences with ambiguous base calls were then removed. The sequence data were then processed using the DADA2 package (Callahan et al., 2016) (version 1.16.0). Sequences were filtered using the function filterAndTrim() with default parameters and the maximum number of expected errors allowed in a read (maxEE) equal to 2. Amplicon sequence variants (ASVs) were generated with default settings and chimeras were removed. Final ASVs were taxonomically classified using the function assignTaxonomy() against the reference dataset Silva version 138.1 (https://www.arb-silva.de/documentation/release-1381/). Nucleotide sequences of ASVs were aligned with MAFFT (Katoh and Standley, 2013) (version 7.48) and a phylogenetic tree was inferred using FastTree (Price et al., 2010) (version 2.1.3) with the GTR + CAT model and the gamma option. ASVs that matched the kingdom of Archaea and Eukaryotes (Chloroplast and Mitochondria) were removed. A total of 783,187 reads were thus obtained.

To account for variable sequencing depths, the abundance table was rarefied to the minimum sequencing depth (27,929 reads) among all samples using the rarefy_even_depth() function in phyloseq package (McMurdie and Holmes, 2013) (version 1.32.0). The differences in the bacterial community composition among soils were determined using weighted UniFrac distance matrices with the UniFrac() function. The weighted UniFrac distance takes into account both the phylogenetic relationship of ASVs and their respective number of reads. Bacterial communities were compared by hierarchical clustering of weighted UniFrac distances using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) with the hclust() function.

The web-based server MicFunPred (http://micfunpred.microdm.net.in/) was used to estimate the functional profiles of the bacterial communities. MicFunPred minimizes false-positive results in comparison to other approaches (Mongad et al., 2021). As a result 5994 KEGG Orthologues (KO) were predicted based on the bacterial ASV sequences and the non-rarefied ASV abundance table. A KO abundance table was then used as input to the web-server MicrobiomeAnalyst in the section shotgun data profiling (Chong et al., 2020). The default options for low count and low variance filters were used: KO identifiers with at least 4 counts in 20% of samples were kept. KO identifiers with variances based on an inter-quantile range below 10% were filtered out. The remaining 5004 KO were scaled using total sum scaling. In order to identify the more abundant KO between soils bacterial communities, differential abundance analysis of KO identifier, using a classical univariate analysis, was followed by an enrichment analysis based on the globaltest algorithm (Goeman et al., 2004). The average number of 16S rRNA gene copies within each of the soil bacterial communities was estimated using the predicted number per genus in MicFunPred and calculating the weighted average based on the samples' relative abundance table. Where there were no predicted 16S rRNA gene copy number, a value of 1 was assumed.

### 2.6. Statistical analyses

Rstudio (Version 1.3.1073 - © 2009–2020 Rstudio, Inc) (RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA) was used for all statistical analysis and plots. Data were transformed to ensure normality and homogeneity of variances where necessary. Non-parametric tests were carried out with the ARTool package (Wobbrock et al., 2011) (version 0.10.7) when transformations did not result in normality. Differences between groups were determined when relevant by pairwise comparisons of the least-square means using adjusted *P*-values (Tukey - implementated in the "emmeans" library (Lenth, 2016) version 1.5.0).

The relationships between heat dissipation profiles and the molecular formulae composition of organic matter or the microbial community composition were assessed using Mantel tests on the respective

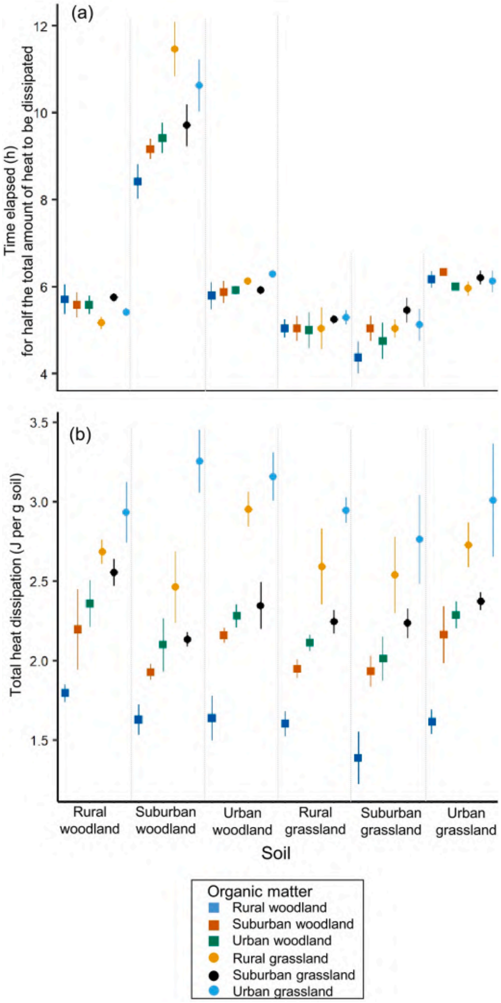

**Fig. 3.** Variables describing the heat dissipation curves. **(a)** Time elapsed for half the total amount of heat to be dissipated, and **(b)** total heat dissipation. Each symbol depicts the mean ± one standard deviation (n = 4) after subtraction of the mean heat dissipation in control soils that only received water.

**Table 1**
**Results of statistical analyses of the two variables that characterised the heat dissipation profiles.** Abbreviation: organic matter (OM).

| Variables | Test | Parameter | Degree of freedom | Test Statistic | *P*-value |
|---|---|---|---|---|---|
| Time elapsed for half the total amount of heat to be dissipated | Analysis of Variance of Aligned Rank Transformed Data | Soil | 5 | F = 186.4989 | $<2.22 \times 10^{-16}$ |
| | | OM | 5 | F = 18.1379 | $4.9705 \times 10^{-13}$ |
| | | interaction OM: Soil | 25 | F = 6.3925 | $3.2681 \times 10^{-12}$ |
| Total heat dissipation | Two-way analysis of variance (ANOVA) | OM (log-transformed data) | 5 | F = 273.271 | $<2 \times 10^{-16}$ |
| | | Soil (log-transformed data) | 5 | F = 15.734 | $1.29 \times 10^{-11}$ |
| | | interaction OM: Soil (log-transformed data) | 25 | F = 2.003 | 0.00774 |

distance matrix with the package vegan (Oksanen et al., 2014) (version 2.6–6) and ade4 (Chessel et al., 2004) (version 1.7–15).

## 3. Results

### 3.1. Temporal and hierarchical pattern of microbial activity in soils

There were clear differences in the dynamics of heat dissipation due to microbial activity among soils and organic matter (Fig. 2). The shape of the curves (i.e. the heat dissipation dynamics) tended to depend on the soil and there was a hierarchy of heat dissipation that depended on the organic matter added. The heat dissipation was generally highest when soils received urban or rural grassland organic matter and lowest when soils received rural or suburban woodland organic matter.

We further characterised the heat dissipation profiles using a combination of two variables: the time elapsed for half the total amount of heat to be dissipated and the total heat dissipation, describing, respectively, the dynamics of heat dissipation and the overall soil microbial activity (Fig. 3). There were significant differences ($P < 0.001$) in the time elapsed for half the total amount of heat to be dissipated (Fig. 3a), with the suburban woodland soil showing relatively late heat dissipation and the suburban and rural grassland soils showing early heat dissipation. The variations in dynamics were also dependent on the organic matter added ($P < 0.001$), but to a lesser extent. Furthermore, there was an interaction between soils and organic matter ($P < 0.001$), suggesting that the changes in metabolic response to the organic matter additions were not constant across soils (Table 1). The grassland organic C stimulated significantly ($P = 0.011$; Student's t-test) more rapid microbial activity than the woodland organic matter (Fig. 3a), but there was no effect of the urban pressure gradient.

No significant differences in the overall microbial activity were apparent between soils (Fig. 3b). However, within each soil, there was a significant hierarchy related to the origin of the organic matter added. The hierarchy was consistent across soils, with the urban grassland organic matter always resulting in higher total heat dissipation ($P < 0.001$) and the rural woodland organic matter producing the lowest dissipation of heat ($P < 0.001$) (Figs. 2 and 3). Furthermore, the total heat dissipation in response to grassland OM additions was significantly greater than that after the addition of woodland OM (Fig. 3b). This suggests that the composition of the organic matter affected the overall metabolic response, regardless of the properties of the soils or of the resident microbial decomposers.

None of the abiotic soil properties (e.g. pH, texture, phosphate content) were significantly linearly correlated with microbial activity after the addition of organic matter.

### 3.2. Composition of microbial communities and organic matter

The composition of the soil microbial communities with the most rapid metabolic response to the organic matter addition (suburban grassland) contained the highest proportion of *Bacteroidia*, while the communities with the slowest response (suburban woodland) had the lowest proportion of *Bacteroidia* (Fig. 4). The relative abundances of *Alphaproteobacteria* and *Actinobacteria* were highest in the suburban woodland, while the proportion of *Gammaproteobacteria* was highest in soils in which heat dissipation occurred rapidly after OM additions. There was a significant negative relationship between the time elapsed for half the total amount of heat to be dissipated and the predicted average 16 rRNA gene copy number within the community (Fig. S1). Furthermore, the prediction of the functional profiles (KEGG

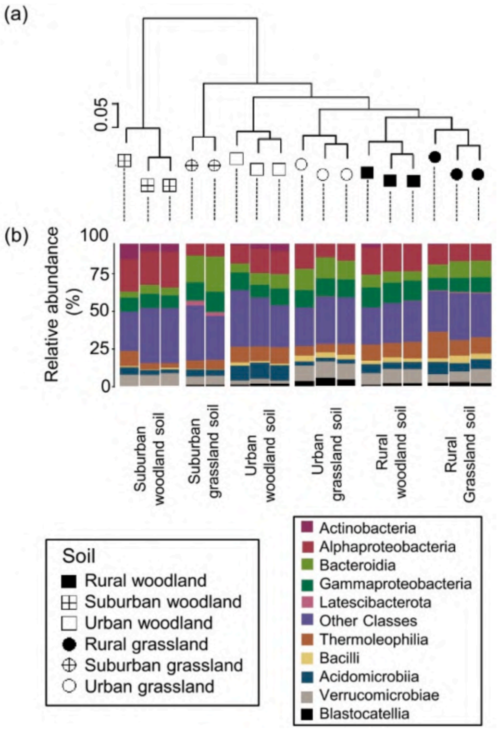

**Fig. 4.** Differences in bacterial community composition between soils. **(a)** Hierarchical clustering of weighted UniFrac distances of rarefied ASVs data using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), and **(b)** taxonomic profiles (at the class level) labeled by soil were ordered based on their position in the UPGMA phenogram.

**Table 2**

**Diversity and energetic return on investment indices of water-soluble organic matter.** Abbreviations: nominal oxidation state of carbon (NOSC), heat of combustion (ΔE), standard state Gibbs energies of oxidation half reactions of organic compounds ($\Delta G^{\circ}_{Cox}$), potential energetic return on investment (ROI).

| Soluble Organic matter | Molecular formulae | | NOSC | | ΔE (J $mmol^{-1}$ of C) | Sum of the intensity weighted NOSC | $\Delta G^{\circ}_{Cox}$ (J $mmol^{-1}$ of C) | ROI ($\Delta E/\Delta G^{\circ}_{Cox}$) |
|---|---|---|---|---|---|---|---|---|
| | Richness | Simpson effective | Richness | Simpson effective | | | | |
| Rural Woodland | 2007 | 139 | 641 | 52 | NA | - 0.30 | 68.75 | NA |
| Suburban Woodland | 2147 | 82 | 699 | 34 | 542.41 | - 0.44 | 72.88 | 7.44 |
| Urban Woodland | 1896 | 57 | 534 | 27 | 567.46 | - 0.47 | 73.65 | 7.70 |
| Rural Grassland | 1978 | 119 | 682 | 44 | 572.25 | - 0.16 | 64.99 | 8.81 |
| Suburban Grassland | 1914 | 75 | 632 | 38 | 555.20 | - 0.33 | 69.80 | 7.95 |
| Urban Grassland | 2011 | 136 | 699 | 55 | 672.46 | - 0.23 | 66.91 | 10.05 |

orthologues) based on the taxonomic composition indicated that the microbial communities that had the earliest heat dissipation phase (the suburban and rural grassland soils; Fig. 3a) were enriched in pathways for the degradation of aromatic compounds (Fig. S2), whereas the soil with the slowest heat dissipation dynamics (the suburban woodland) was enriched in starch and sucrose metabolism (Fig. S3).

The organic matter from each of the soils was composed of the same compound classes in roughly the same proportions (Fig. S4), but the compositional profiles (Fig. S5) and diversities of molecular formulae (Table 2) were different. The number of molecular formulae in each organic matter ranged from 1896 in the urban woodland to 2147 in the suburban woodland. There were no significant differences in the number of molecular formulae between woodland and grassland organic matter. This translated into different NOSC profiles (Fig. 5) and diversities of molecular formulae and NOSC (Table 2). The median NOSC values of the organic C from all the soils were negative, ranging between −0.35 in the urban woodland and −0.14 in the rural grassland. The NOSC profiles of the organic C from the urban woodland and the rural grassland were significantly different ($P = 0.015$; Kolmogorov-Smirnov test). The richness of NOSC ranged from 534 in the urban woodland to 699 in the suburban woodland and the suburban grassland (Table 2). There were no significant differences in NOSC richness between woodland and grassland soil organic matter and NOSC richness was not affected by urban pressure (data not shown). None of the soil properties measured were related to NOSC richness, with the exception of total P content, which showed a significant negative relationship (Fig. S6).

### 3.3. *Relationships between heat dissipation, microbial and organic matter profiles*

We then determined the extent of the relationship between the heat dissipation dynamics with either the composition of soil bacterial communities or with the composition of the added organic matter, using Mantel tests. These showed that dynamics of heat dissipation were more closely related to bacterial community composition than to the composition of the organic matter (Table 3). The suburban woodland soil not only had the slowest heat dissipation dynamics, but also showed the most divergent bacterial community composition (Fig. 4).

None of the metrics used to describe the organic matter (molecular or NOSC profiles and diversities) were related to the heat dissipation dynamics nor to the overall heat dissipation. The organic matter energy contents was significantly positively related to the total heat dissipated in three of the soils (Fig. S7) and the intensity weighted average molecular formulae C:N ratios was significantly negatively related to the total heat dissipation in five of the soils (Fig. S8). The intensity weighted average molecular formulae C:N ratios of the grassland organic were significantly lower than those of the woodland organic matter (Fig. S8; Table 2).

### 3.4. *Energetic return on investment (ROI) of water-soluble organic matter*

There were strong, significant positive relationships between the potential energetic return on investment that soil microorganisms can obtain when processing the organic matter and the overall heat dissipation, across all the six soils (Fig. 6). The potential ROI that could be obtained from grassland OM was always higher than in the woodland OM.

## 4. Discussion

### 4.1. *Factors controlling microbial transformation of organic matter*

It has been suggested that microbial decomposition of available organic matter is controlled by the quality (composition, energy content) of the organic matter (Kallenbach et al., 2015; Takriti et al., 2018) or by the properties of the microbial communities themselves (Strickland et al., 2009; Fraser et al., 2016; Nunan et al., 2017). This study suggests that total decomposition is dependent on the energetic properties of the available organic matter and that the decomposition dynamics depend on the properties of the microbial communities. In view of the effects that soil properties have on microbial communities (Liu et al., 2018; Rasmussen et al., 2018; Suriyavirun et al., 2019), they might be expected to also affect the decomposition of the added organic matter. This was not the case however. None of soil properties measured (pH, texture, P content, total organic C content, total N content) were significantly correlated with the indices of heat dissipation. The lack of relationship may be due to the fact that the soil properties did not vary widely and therefore would not have had differential effects on microbial responses to organic matter additions.

The grassland soil organic matter tended to be decomposed more rapidly by microbial communities across soils and resulted in higher total activity, meaning that the first hypothesis was accepted. The results confirm what is known from the literature which suggests that grassland organic matter contains more labile forms than woodland soil organic matter (Chantigny, 2003). Furthermore, the C:N ratios of the grassland organic matter were lower than those of the woodland organic matter, suggesting that soils receiving woodland organic matter may have been N limited. However, the total heat dissipation was always more closely related to the potential ROI (Fig. 6) than to the intensity weighted average molecular formulae C:N ratios of the organic matter additions (Fig. S8), suggesting that it is the energetic properties of the organic matter additions that determined total heat dissipation rather than the N content.

### 4.2. *Temporal pattern of microbial activity in soil*

The data presented here suggest that the dynamics of organic matter consumption is more related to the taxonomic composition of bacterial communities than to the composition of the substrate, at least in the case of short-term dynamics where abiotic constraints are reduced.

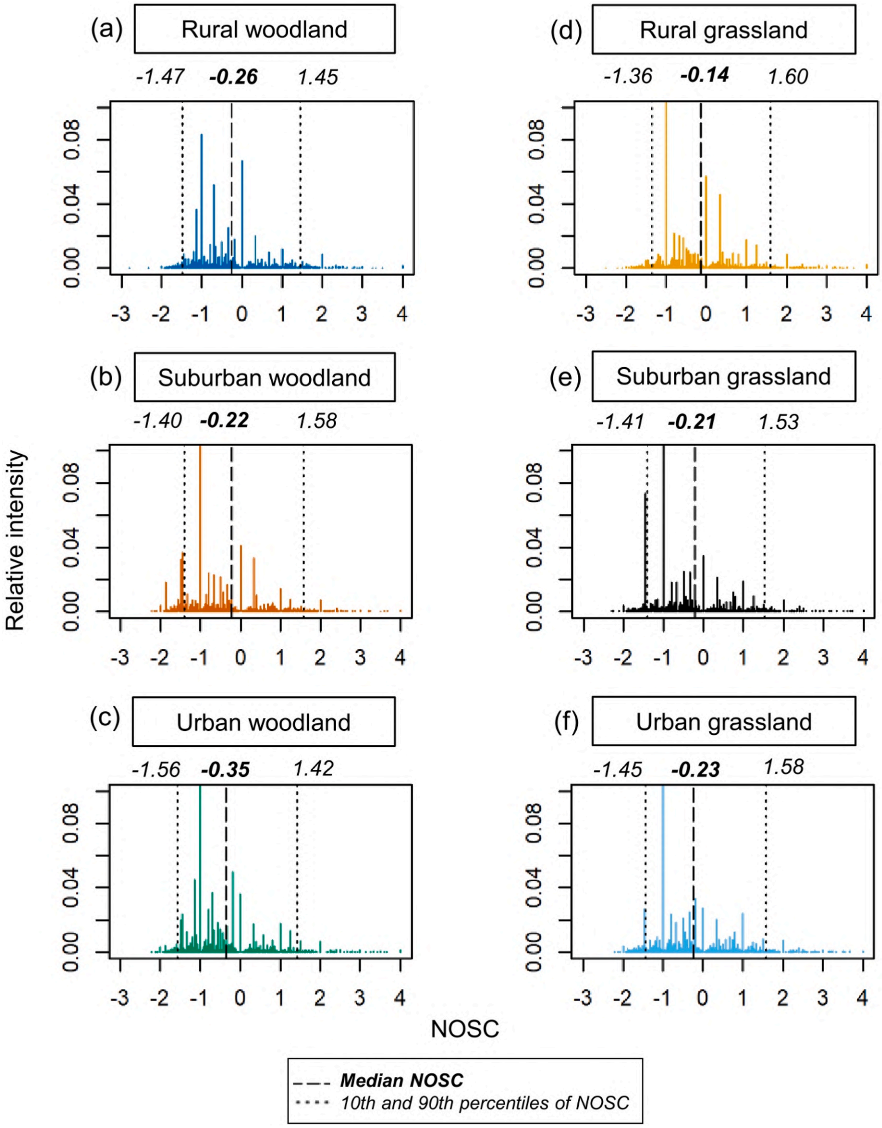

**Fig. 5.** Distribution of the relative intensities of nominal oxidation state of carbon (NOSC) in water-soluble organic matter (OM). **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soluble OM. A Kolmogorov-Smirnov test on the NOSC data of each soluble organic matter indicated that the distribution of NOSC from the urban woodland was significantly different from that of the rural grassland ($D = 0.090$, $P = 0.015$).

Furthermore, the urban pressure gradient did not influence the dynamics of heat dissipation which led us to reject the second hypothesis. It should be noted that the range of soil properties in this study was limited. Had it been greater, then microbial activity might have been differentially constrained by some of these properties (e.g. soil pH, nutrient availability), thus changing the relationships observed here. There are a number of possible explanations for the differences in metabolic dynamics displayed by the microbial communities.

The first possible explanation is that the microbial communities in soils that responded rapidly to the addition of organic matter had different life history strategies (i.e. the tradeoffs between growth, survival and reproduction) from those in soils that responded more slowly.

**Table 3**
Mantel tests of heat dissipation profiles with soil bacterial taxonomic composition or with water-soluble organic matter molecular composition. Abbreviations: organic matter (OM).

| Dissimilarity indices | Dissimilarity indices of heat dissipation profiles[c] | Mantel R | P-value |
|---|---|---|---|
| Soil bacterial taxonomic composition[a] | Rural woodland OM | 0.9180 | 0.01 |
| | Suburban woodland OM | 0.8671 | 0.07 |
| | Urban woodland OM | 0.9068 | 0.02 |
| | Rural grassland OM | 0.8881 | 0.07 |
| | Suburban grassland OM | 0.8864 | 0.11 |
| | Urban grassland OM | 0.8972 | 0.07 |
| Soluble OM molecular composition[b] | Rural woodland soil | 0.5174 | 0.13 |
| | Suburban woodland soil | 0.2879 | 0.22 |
| | Urban woodland soil | 0.5515 | 0.08 |
| | Rural grassland soil | 0.4398 | 0.12 |
| | Suburban grassland soil | 0.6014 | 0.08 |
| | Urban grassland soil | 0.5959 | 0.09 |

[a] Weighted UniFrac dissimilarity index calculated with rarefied ASVs data between each soil.

[b] Bray-Curtis dissimilarity index calculated with normalised FT-ICR-MS data between each organic matter.

[c] Bray-Curtis dissimilarity indices calculated with normalised heat dissipation rates data for either from one organic matter between each soil or from one soil between each organic matter.

The soils with more rapid heat dissipation dynamics harboured higher relative abundances of *Bacteroidia* and *Gammaproteobacteria*, both of which are recognised to contain many copiotrophs (Fierer et al., 2007; Shrestha et al., 2007). Copiotrophs generally contain a greater number of rRNA operon copies than oligotrophs (Fierer et al., 2007), which allows them to respond rapidly to resource pulses and to thrive under resource replete conditions (Li et al., 2019; Langer et al., 2004). *Alphaproteobacteria* and *Actinobacteria,* both of which were relatively abundant in the soil with the slowest heat dissipation dynamics, are known to be dominated by phylotypes with low 16S rRNA operon copy numbers (DeAngelis et al., 2015; Shrestha et al., 2007). Lower 16S rRNA operon copy numbers in cells tends to lead to a slower microbial growth rates in response to pulses of resources (Li et al., 2019).

The second possible explanation is that metabolic pathways (i.e. the sequence of chemical reactions catalyzed by enzymes) are distinct at the community level. Here, the prediction of the functional profiles (KEGG orthologues) based on the taxonomic composition suggested that there were indeed contrasting metabolic pathway profiles. Metabolic pathways associated with lower enzyme demand can allocate free energy to other cellular processes (Flamholz et al., 2013; Wortel et al., 2018), such as growth, thus driving the observed temporal variation in microbial activity across soils in our study. However, this avenue would have to be investigated further.

A third possible explanation is that the size of the microbial biomass was greater in the soils that responded more rapidly to the additions of organic matter. The size of the soil microbial biomass has been previously shown to impact the dynamics of soil respiratory responses (Fraser et al., 2016). Although we did not measure the microbial biomass, the lack of relationship between heat dissipation and the organic C content of the soils suggests that the size of the microbial biomass was not a factor in determining the temporal patterns of microbial activity. The microbial biomass of soil is generally closely related to the organic C content (Anderson and Domsch, 1989).

### 4.3. Hierarchical pattern of microbial activity in soils

Our data suggest that neither the composition of the organic matter, the overall energy availability, nor the diversity of molecular compounds and NOSC, directly determine the overall metabolic response of microbial communities when consuming organic matter. This may be viewed as a surprising conclusion to come to, as the oxidation of molecular species with higher NOSC is more favorable from a thermodynamic point of view (LaRowe and Van Cappellen, 2011). However, microbial communities have to make metabolic investments (e.g. production of enzymes and transport proteins) in order to acquire resources (Smith and Chapman, 2010; Malik et al., 2020) and the magnitude of these investments depends on both the composition of the organic matter that is available (Allison and Vitousek, 2005; LaRowe and Amend, 2016) and the composition of the microbial biomass (LaRowe and Amend, 2016). The metabolic response of microbial decomposers is therefore likely to be related to the energetic return on investment that they get from the available organic resources rather than the overall energy availability or the molecular diversity. This implies that the metabolic response is more likely to be related to a combination of the overall energy availability and the ease with which it can be used by microbial communities.

The absence of a correlation between microbial heat dissipation and molecular diversity or NOSC (Fig. 3 and Table 3) led us to reject the third hypothesis, namely that the molecular heterogeneity of the added organic matter would be negatively related to microbial metabolism. This suggests that microbial communities did not incur additional costs associated with substrate diversity. This may be because the microbial communities were able to maintain a sufficiently large range of catabolic pathways to consume the diverse substrate available. The lack of a relationship tends to contradict the suggestion by Lehmann et al. (2020) that the persistence of soil organic matter can be explained by its molecular heterogeneity. These authors suggest however, that it is low concentrations of heterogeneous organic matter that limit decomposition. The concentrations used in this experiment were likely much higher than those that were proposed to lead to organic C persistence.

### 4.4. Energetic return on investment (ROI)

The highly significant relationships between the potential energetic return on investment and the actual heat dissipation across all of the soils confirms the results of Garayburu-Caruso et al. (2020) and allows us to accept the fourth hypothesis of the study, namely that the greater the estimated potential energetic return on investment available to microbial decomposers in organic matter, the greater the metabolic activity. What might the biological mechanisms underlying this relationship be? In order to acquire energy during the mineralisation of organic C, decomposers must first remove electrons from the substrates and, the higher the $\Delta G^{\circ}_{Cox}$ of the organic matter, the more energy is required to remove them (LaRowe and Van Cappellen, 2011). We suggest that the higher energetic costs associated with removing such electrons translate into higher metabolic costs for microbial decomposers (Fig. 1). The extra metabolic costs may be due to the need to produce enzymes in greater quantity (Noor et al., 2016), to make use of additional cofactors (Sousa et al., 2020) or to produce enzymes with larger catalytic domains (Arcus et al., 2016). Arcus et al. (2016) surveyed a range of enzymes (hydrolases, esterases, decarboxylases, isomerases) and found that, within each enzyme group, larger enzyme catalytic domains were required to catalyse more difficult reactions (i.e. slower reactions when not in the presence of enzymes). Protein synthesis, including the synthesis of enzymes, is a major component of microbial cells' energy expenditure (Lane and Martin, 2010). Therefore, any increase in the number or size of enzymes required to catalyse a reaction is likely to lead to increased metabolic costs to microbial cells.

It is interesting to note that although there was a relationship between the thermodynamic favorability of organic substrate and microbial respiration in sediments under C limiting conditions, this was not the case when C was not limiting (Garayburu-Caruso et al., 2020). The authors suggested that N limitation regulated respiration under these conditions. Although the availability of N may have affected microbial activity here, their was no relationship between the total N availability in the added organic matter, measured as the C:N ratio by an elemental analyser (Table S2), and the overall heat dissipation suggesting that N

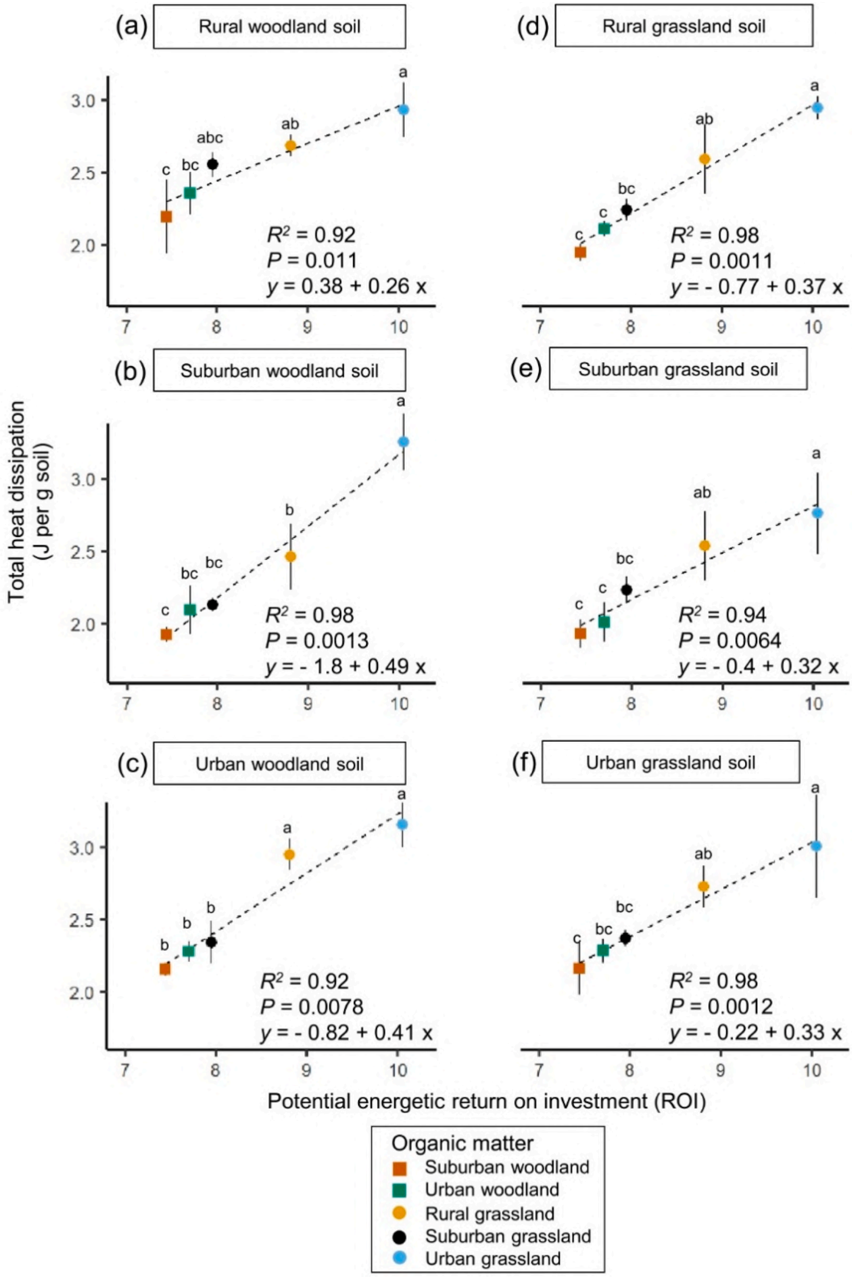

**Fig. 6.** Relationships between the total heat dissipation and the potential energetic return on investment of water-soluble organic matter. **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soils. Each symbol represents the mean ± one standard deviation of the total heat dissipated (n = 4). Differences were determined using a two-way ANOVA and pairwise comparisons of the least-squares means using adjusted *P*-values (Tukey).

limitation was not a major factor.

The data suggest that, when microbial activity is not constrained by other factors (e.g. N availability, physical access to substrate), then it is related to the amount of energy available in substrate and the ease with which the energy can be extracted by microbial decomposers. In other words, the overall microbial activity is related to the forms of energy available to microbial communities.

### 4.5. *Potential contribution to modelling C dynamics*

The metabolic cost associated with the consumption of different types of organic substrate is implicitly represented in the continuum of C qualities model (Bosatta and Ågren, 1999). The model assumes that more energy dense organic substrates, or high-energy compounds, are processed through longer metabolic pathways. As each additional step in a metabolic pathway requires additional enzymes, the metabolic cost is increased (Niebel et al., 2019). However, the model is empirical rather than explicit and therefore cannot account for the interactions between organic substrate and decomposer. By incorporating the concept, using metrics such as those proposed here, it may be possible to better account for the effects of both microbial and organic matter changes on soil C dynamics.

### 4.6. *Potential limits of the study*

One of the underlying assumptions of the study is that there were aerobic conditions throughout incubations, thus ensuring that oxygen was the terminal electron acceptor. Were the conditions anaerobic, then other terminal electron acceptors (e.g. nitrate, pyrolusite ($MnO_2$), goethite (FeOOH), sulfate) would have been used and the net energy available to the microbial communities from the oxidation of the organic matter would have been lower than the maximum potential energy resulting from aerobic respiration ($\Delta$E) estimated by bomb calorimetry. This is due to the fact that lower amounts of energy are released during the reduction of terminal electron acceptors others than oxygen (Amend and LaRowe, 2019). Although oxygen levels were not measured during the incubation, it is safe to assume that the conditions remained aerobic. The incubations were quite short and the soil moisture levels were optimal for aerobic activity.

## 5. Conclusion

The major conclusion to be drawn from this study is that soil C dynamics can only be fully understood through the prism of interactions between organic substrate and microbial decomposers. Contrasting microbial communities displayed relatively large variations in heat dissipation dynamics, while the energetic properties of the organic substrate affected the total metabolic response. We therefore propose that the potential energetic return on investment microbial community can achieve when transforming soil organic matter is a relevant indicator for predicting total microbial activity in hotspots. The potential energetic return on investment that microbial communities could achieve when consuming the added organic matter did not depend on the urban pressure gradient or on the land-use type. As a result, neither the urban pressure gradient nor the land-use type affected the total microbial activity in response to the organic matter amendments. However, these results would need to be confirmed with a broader set of soils.

## Author contributions

Conceptualization: A.M.H. and N.N. Methodology: A.M.H., NN, and L.J.P.D. Resources: N.N., A.M.H., C.P., and J.L. Investigation: L.J.P.D., C.P., and J.L. Formal analysis: L.J.P.D. and N.N. Visualization: L.J.P.D. Validation: N.N., A.M.H., J.L., C.P. and L.J.P.D. Funding acquisition: A.M.H., N.N., C.P., and L.A. Project administration: A.M.H. and N.N. Supervision: N.N., A.M.H., L.F., and L.A. Writing – original draft: N.N., L.J.P.D., A.M.H., C.P., and J.L. Writing – review & editing: N.N., L.J.P.D, A.M.H.

## 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.

## Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and the Supplementary Materials. 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 a public repository entitled zenodo (https://zenodo.org/record/5547311) (Creative Commons Attribution 4.0 International). The nucleotide reads have been deposited to NCBI under the BioProject PRJNA724026; sample accession numbers are: SAMN18839354 to SAMN18839371.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108800.

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# Supplementary Materials for

# Potential energetic return on investment positively correlated with overall soil microbial activity

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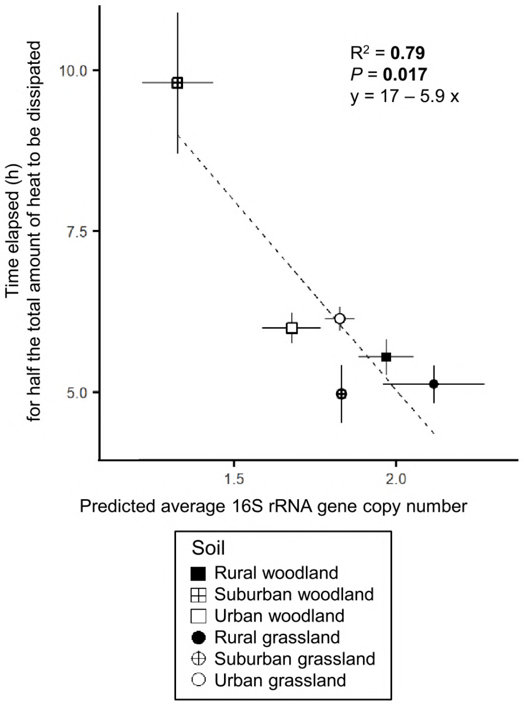

**Fig. S1. Relationship between the time elapsed for half the total amount of heat to be dissipated and the predicted average 16S rRNA gene copy number within the soil bacterial community.** Each symbol represents the mean ± one standard deviation of the time elapsed for half the total amount of heat to be dissipated (n = 4) and the predicted average 16S rRNA gene copy number (n = 3, except for the suburban grassland soil where n = 2).

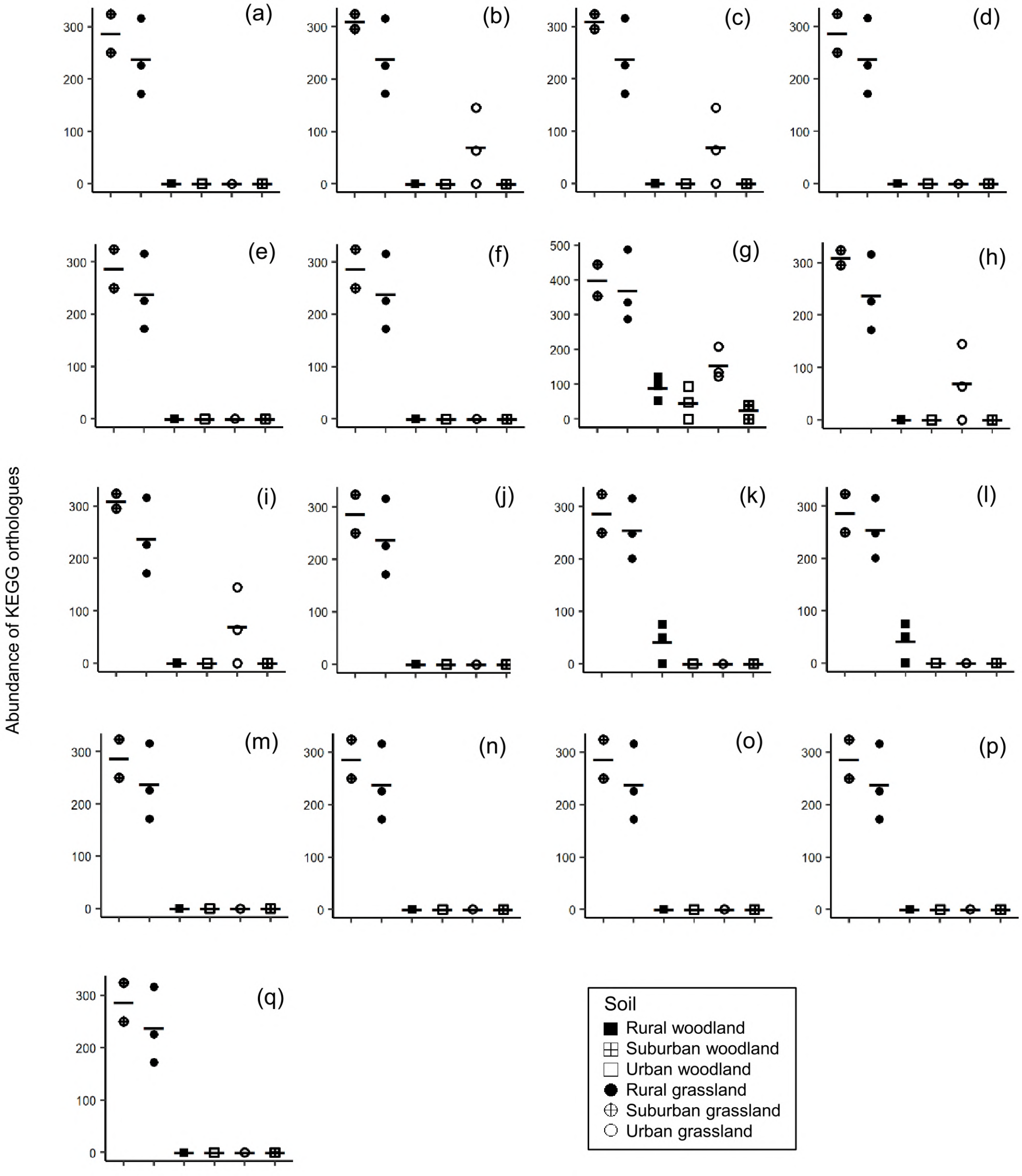

**Fig. S2. Abundance of KEGG orthologues involved in the degradation of aromatic compounds that were significantly enriched in the bacterial community of different soils.** Each dash symbol represents the mean of the abundance of KEGG orthologues within each soil (n = 3, except for the suburban grassland soil where n = 2). **(a)** K04108, **(b)** K04109, **(c)** K04112, **(d)** K04113, **(e)** K04114, **(f)** K04115, **(g)** K07537, **(h)** K07538, **(i)** K07539, **(j)** K07540, **(k)** K07543, **(l)** K07544, **(m)** K07545, **(n)** K07547, **(o)** K07548, **(p)** K07549, **(q)** K07550.

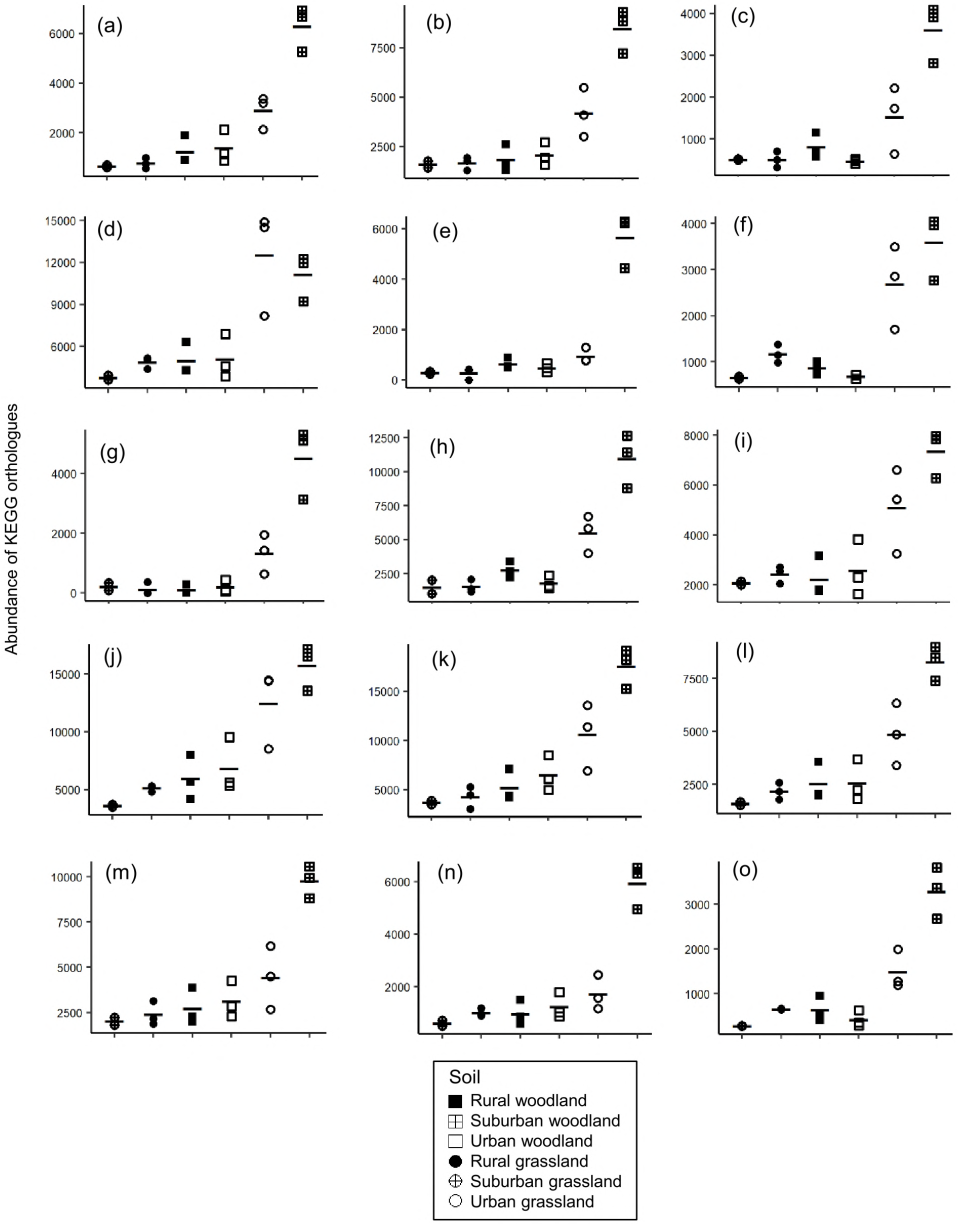

**Fig. S3. Abundance of KEGG orthologues involved in the starch and sucrose metabolism that were significantly enriched in the bacterial community of different soils.** Each dash symbol represents the mean of the abundance of KEGG orthologues within each soil (n = 3, except for the suburban grassland soil where n = 2). **(a)** K00694, **(b)** K00705, **(b)** K01178, **(d)** K01179, **(e)** K01184, **(f)** K01193, **(g)** K01195, **(h)** K01198, **(i)** K01236, **(j)** K02438, **(k)** K05343, **(l)** K06044, **(m)** K16147, **(n)** K16148, **(o)** K16149.

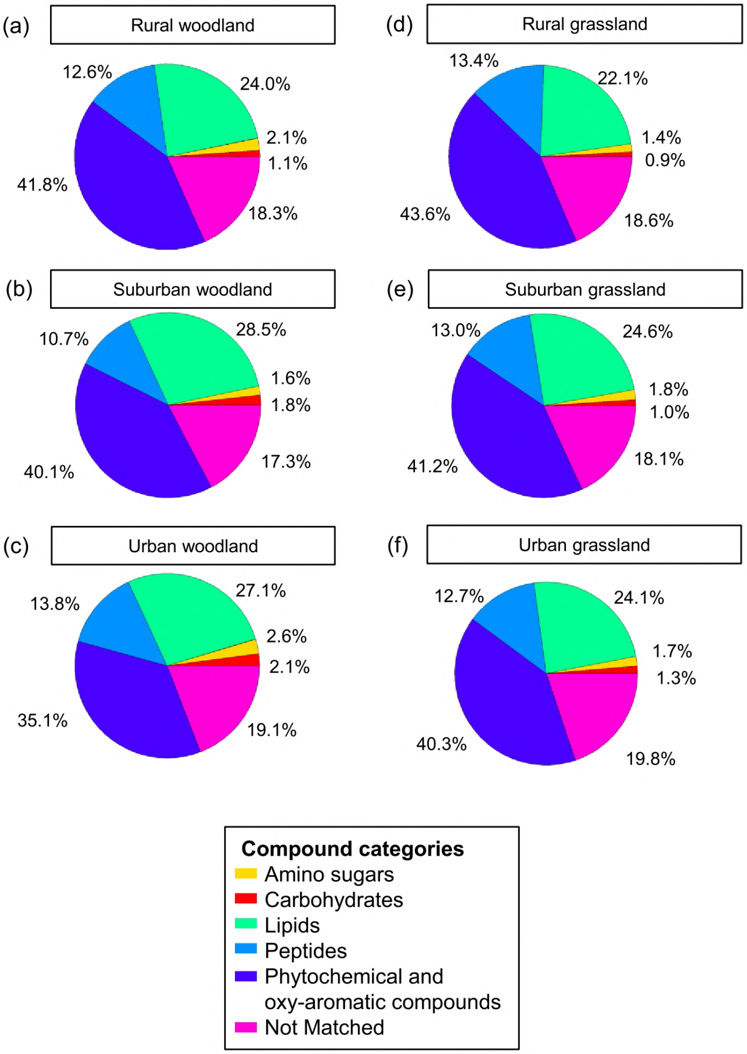

**Fig. S4. Pie diagrams of the relative abundance (%) of the compound categories in soluble organic matters.** The compound categories are defined by the multidimensional stoichiometric compound classification for soluble organic matters (OM) from **(a)** rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, **(f)** urban grassland. Molecular formula that did not matched to any of the molecular compound categories are shown in pink.

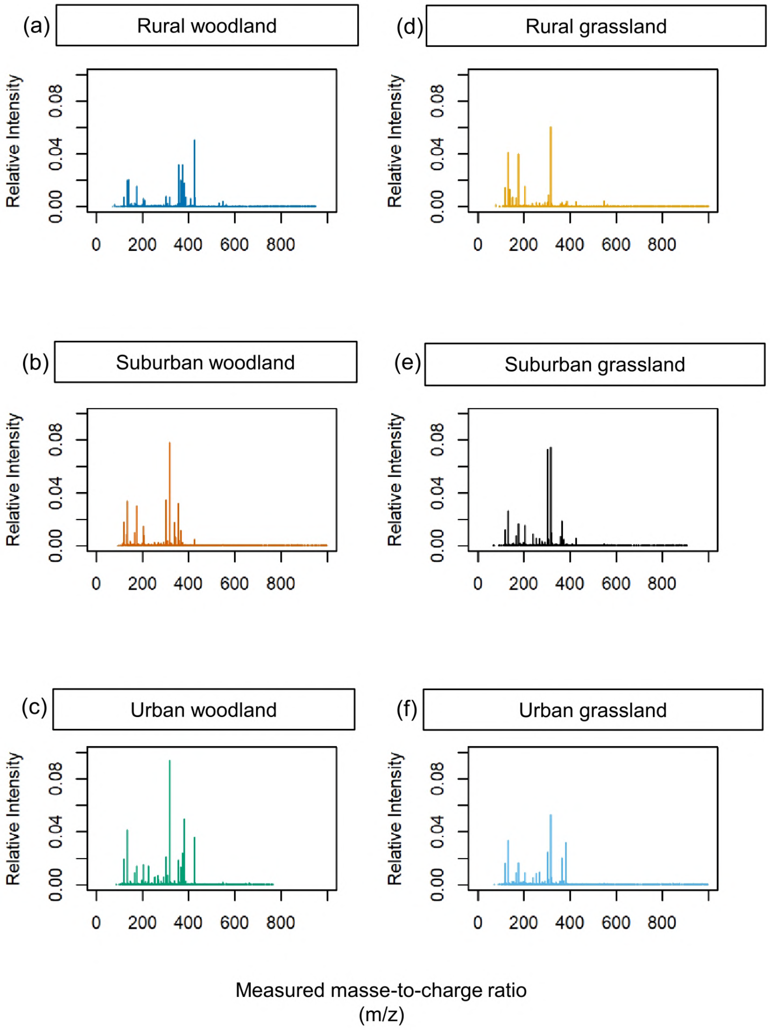

**Fig. S5. Distribution of the relative intensities of measured mass-to-charge ratio (m/z) by FT-ICR-MS in soluble organic matters (OM).** Soluble organic matters (OM) are from **(a)** rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, **(f)** urban grassland.

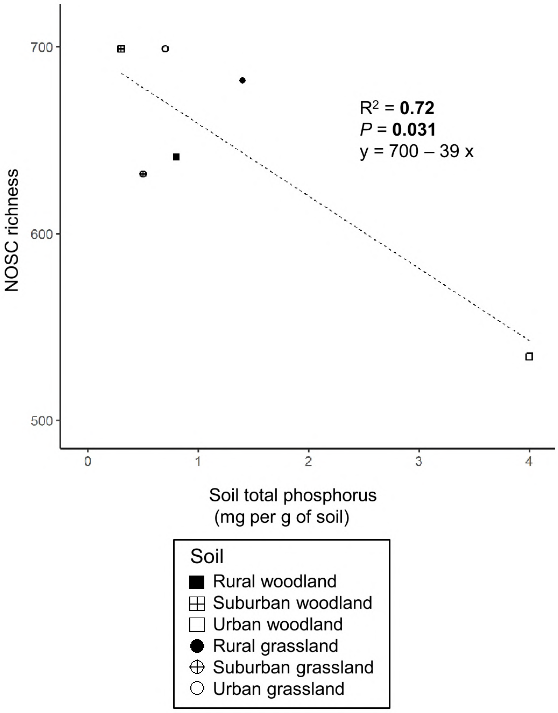

**Fig. S6. Relationships between the NOSC richness and the soil total phosphorus.** Each symbol represents the value of the NOSC richness in the soluble OM from different soil (n=1) and the total phosphorus content in each of these soils (n=1).

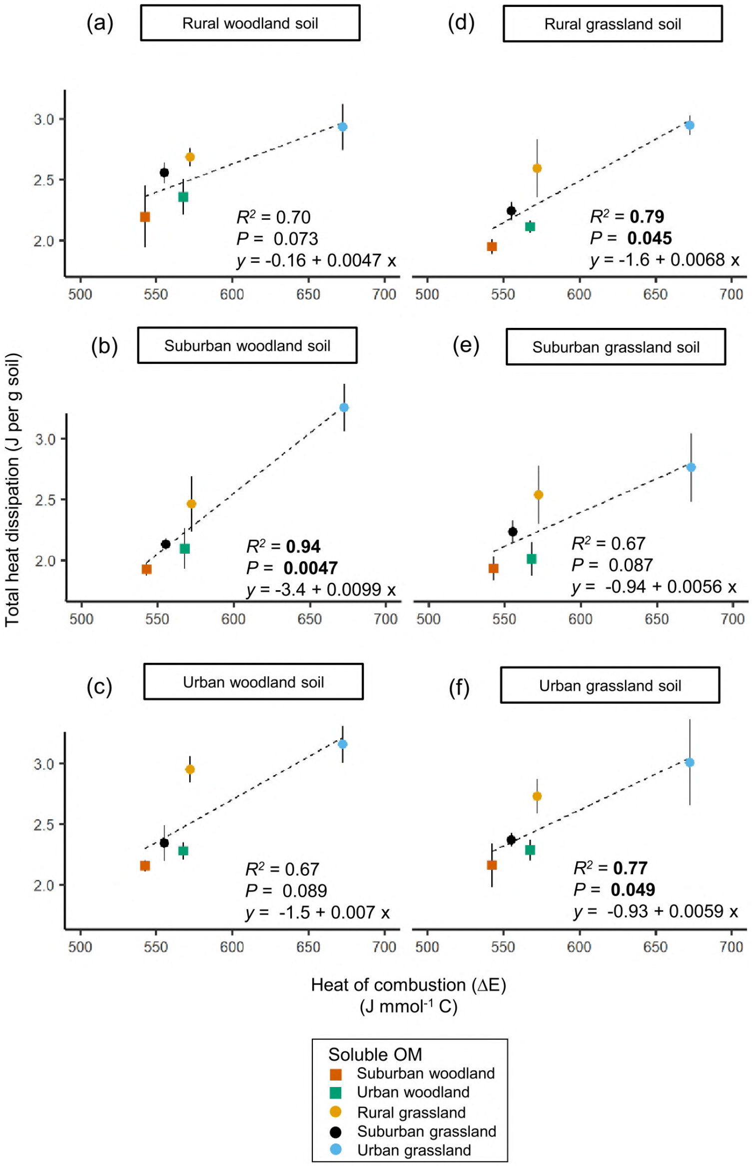

**Fig. S7. Relationships between the total heat dissipation and the heat of combustion (ΔE).** Each relationship is determined for **(a)** rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland or **(f)** urban grassland soils. Each symbol represents the mean ± one standard deviation of the total heat dissipation (n = 4) and the value of heat of combustion (n=1) for each soluble organic matter.

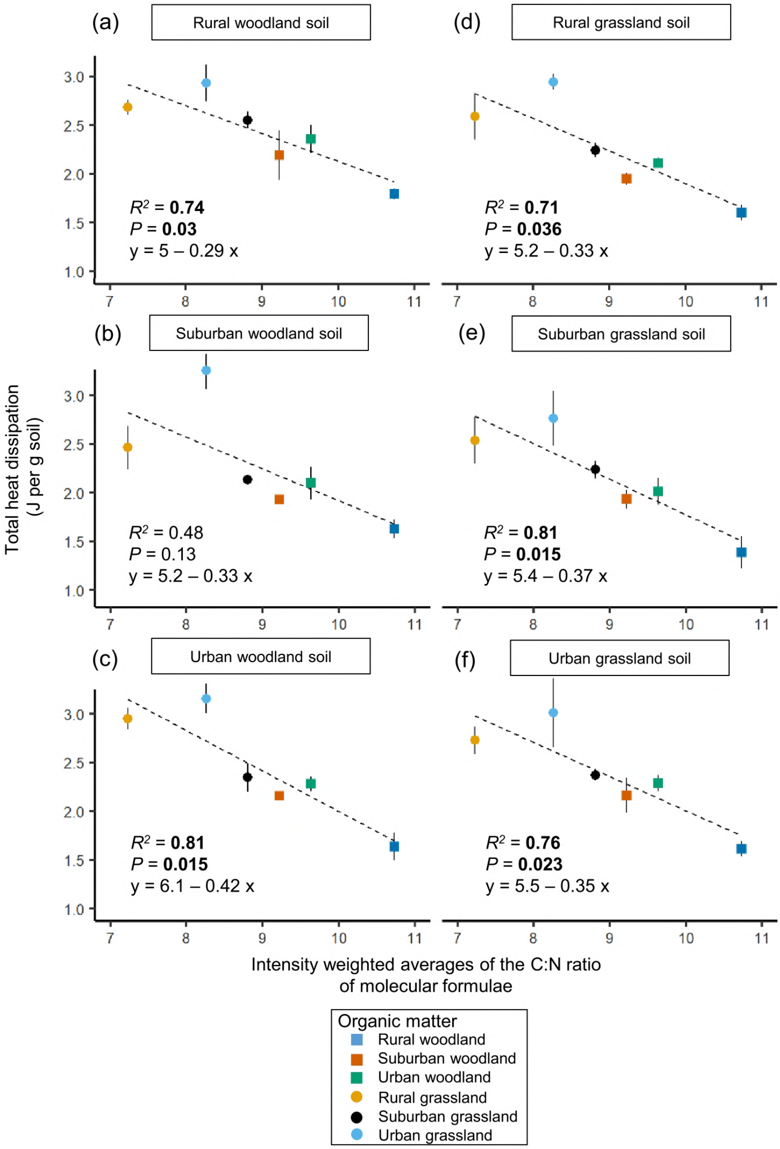

**Fig. S8. Relationships between the total heat dissipation and the intensity weighted averages of the C:N ratio.** Each relationship is determined for **(a)** rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland or **(f)** urban grassland soils. Each symbol represents the mean ± one standard deviation of the total heat dissipation (n = 4). The intensity weighted averages of the C:N ratio (n=1) of each soluble organic matter was calculated as the inverse of the N:C ratio determined by ultra high resolution mass spectrometry, in order to prevent calculation issues when no N was in a molecular formula.

## Table S1. Soil characterization.

| Site | Total organic C* (mg of C per g of soil) | Total N† (mg of N per g of soil) | Ratio C:N† | Water content‡ (mg of $H_2O$ per g of soil) | Total phosphorus§ (mg per g of soil) | pH§ (H2O) | Clay content§ (%) | Sand content§ (%) | Soil texture§ | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rural woodland | 21.0 $^{E}$ ± 0.1 | 1.8 $^{F}$ ± 0.1 | 11.1 $^{E}$ ± 0.1 | 240.3 | 0.8 | 7.2 | 10.7 | 67.6 | Sandy Loam | 48.967306°N | 3.024923°E |
| Suburban woodland | 38.4 $^{B}$ ± 0.1 | 1.9 $^{E}$ ± 0.1 | 19.3 $^{C}$ ± 0.1 | 131.3 | 0.3 | 7.3 | 14.7 | 30.1 | Silt Loam | 48.693488°N | 2.138752°E |
| Urban woodland | 70.1 $^{A}$ ± 0.6 | 3.2 $^{B}$ ± 0.1 | 21.4 $^{A}$ ± 0.1 | 183.8 | 4.0 | 7.5 | 18.5 | 54.7 | Sandy Loam | 48.876425°N | 2.381111°E |
| Rural grassland | 107.7 $^{A}$ ± 25.6 | 4.7 $^{A}$ ± 0.1 | 19.5 $^{B}$ ± 0.1 | 182.7 | 1.4 | 7.3 | 13.2 | 58.5 | Sandy Loam | 48.952708°N | 3.013758°E |
| Suburban grassland | 26.7 $^{D}$ ± 0.3 | 2.4 $^{C}$ ± 0.1 | 10.9 $^{F}$ ± 0.1 | 279.8 | 0.5 | 7.1 | 17.3 | 59.0 | Sandy Loam | 48.699208°N | 2.144033°E |
| Urban grassland | 29.7 $^{C}$ ± 0.3 | 2.2 $^{D}$ ± 0.1 | 13.4 $^{D}$ ± 0.1 | 194.9 | 0.7 | 7.7 | 23.8 | 19.9 | Silt Loam | 48.870783°N | 2.383263°E |

* Total organic carbon content of soils measured with an elementary analyser (n=3). Means followed by a similar letter are not significantly different among soils at $P \leq 0.05$ as determined, after inverse-transforming the data, by Welch's ANOVA for unequal variances and Games-Howell post-hoc test. † Total nitrogen content and ratio C:N of soils determined by an elementary analyser (n=3). Medians followed by a similar letter are not significantly different among soils at $P \leq 0.05$ as determined by non-parametric Kruskal-Wallis test and Criterium Fisher’s least significant difference post-hoc test. ‡ Water content of soils at a matric potential of -0.033 MPa during the incubation at 25 °C during 4 days before the microcalorimetric measurements (n=1). § Data obtained from the same sites by Foti et al. (2017) but not the same sampling.

**Table S2. Water-soluble organic matter characterization. Abbreviation:** organic matter (OM).

| OM | Total organic C* (µg of C per mg of soluble OM) | Total N† (µg of N per mg of soluble OM) | C:N ratio‡ | Intensity weighted averages of Mass-to-charge ratio (m/z) § | Intensity weighted averages of C:N ratio § |
|---|---|---|---|---|---|
| Rural woodland | 247 $^{A}$ ± 5 | 28 $^{C}$ ± 2 | 9 $^{AB}$ ± 0.4 | 414.8228 | 10.7308 |
| Suburban woodland | 230 $^{A}$ ± 21 | 20 $^{BC}$ ± 5 | 12 $^{AB}$ ± 2.3 | 395.7671 | 9.2192 |
| Urban woodland | 161 $^{B}$ ± 4 | 14 $^{AB}$ ± 1 | 12 $^{A}$ ± 0.3 | 340.6191 | 9.6342 |
| Rural grassland | 206 $^{A}$ ± 23 | 20 $^{C}$ ± 2 | 10 $^{B}$ ± 0.3 | 412.0052 | 7.2302 |
| Suburban grassland | 139 $^{BC}$ ± 16 | 14 $^{A}$ ± 2 | 10 $^{B}$ ± 0.1 | 392.4914 | 8.8060 |
| Urban grassland | 126 $^{C}$ ± 9 | 13 $^{A}$ ± 1 | 10 $^{B}$ ± 0.2 | 418.7269 | 8.2611 |

* Total organic carbon content of soils measured with an elementary analyser (n=3). Means followed by a similar letter are not significantly different among soils at $P \leq 0.05$ as determined, after log-transforming the data, by one way ANOVA for equal variances and Tukey's HSD post-hoc test. † Total nitrogen content of soils measured with an elementary analyser (n=3). Means followed by a similar letter are not significantly different among soils at $P \leq 0.05$ as determined, after inverse-transforming the data, by one way ANOVA for equal variances and Tukey's HSD post-hoc test. ‡ Ratio C:N of soils measured with an elementary analyser (n=3). Means followed by a similar letter are not significantly different among soils at $P \leq 0.05$ as determined, after applying the following transformation to the data ($-1 \times (y^{-3})$), by Welch's ANOVA for unequal variances and Games-Howell post-hoc test. **§** Intensity weighted averages of the mass-to-charge ratio and the inverse ratio between stoichiometric number of nitrogen and carbon in molecular formulae (< 1000 Da) determined by ultra high resolution mass spectrometry.

# III

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RESEARCH ARTICLE

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

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**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

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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

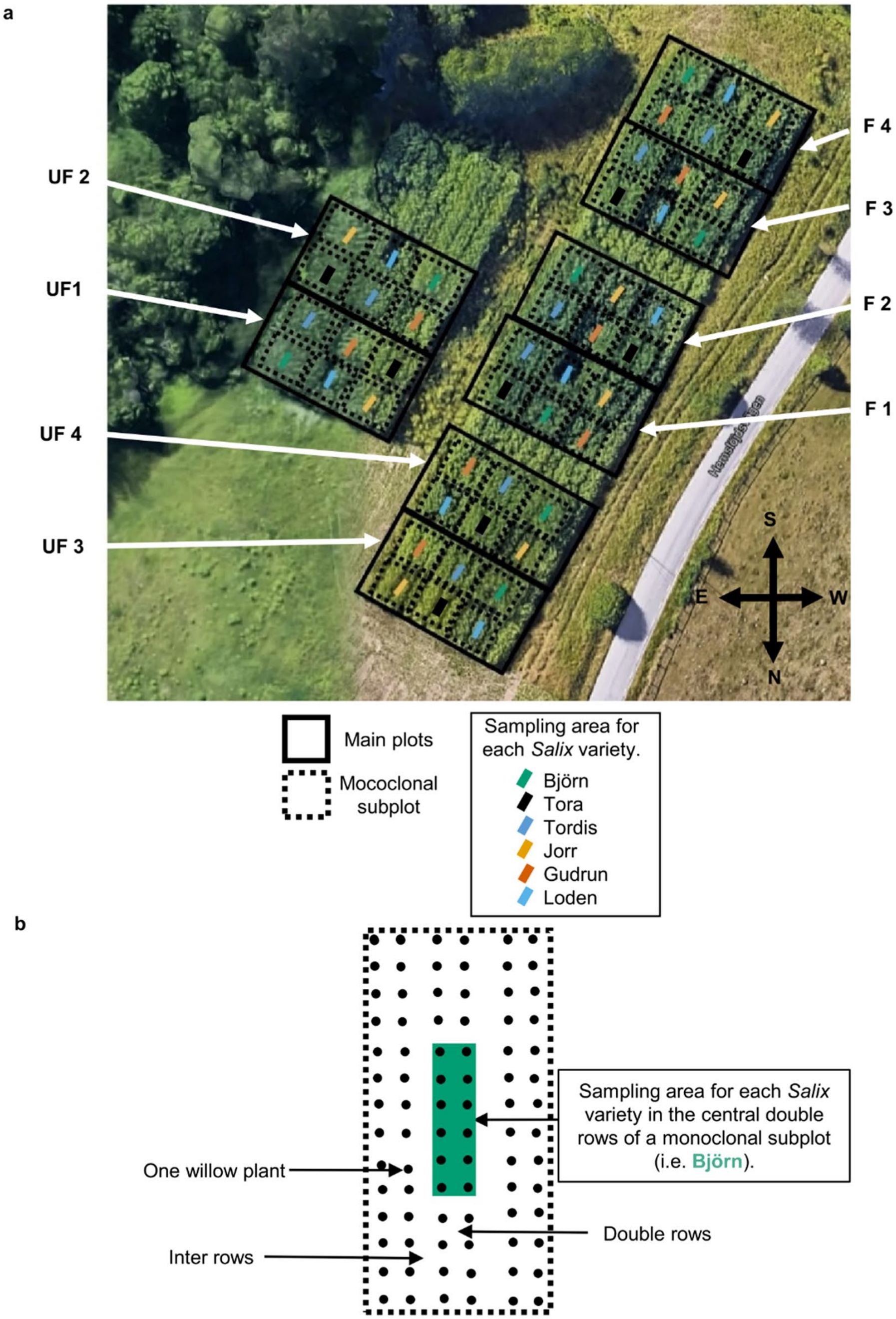
We used a field trial located in central Sweden (59°48′22″N 17°40′24″E). Within this field trial, willow varieties (*Salix* spp.), across which taxonomic distance varied, were cultivated as short rotation coppice on a former arable cropland. The climate is temperate oceanic, and the soil is classified 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 $6.75 \times 7$ 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).

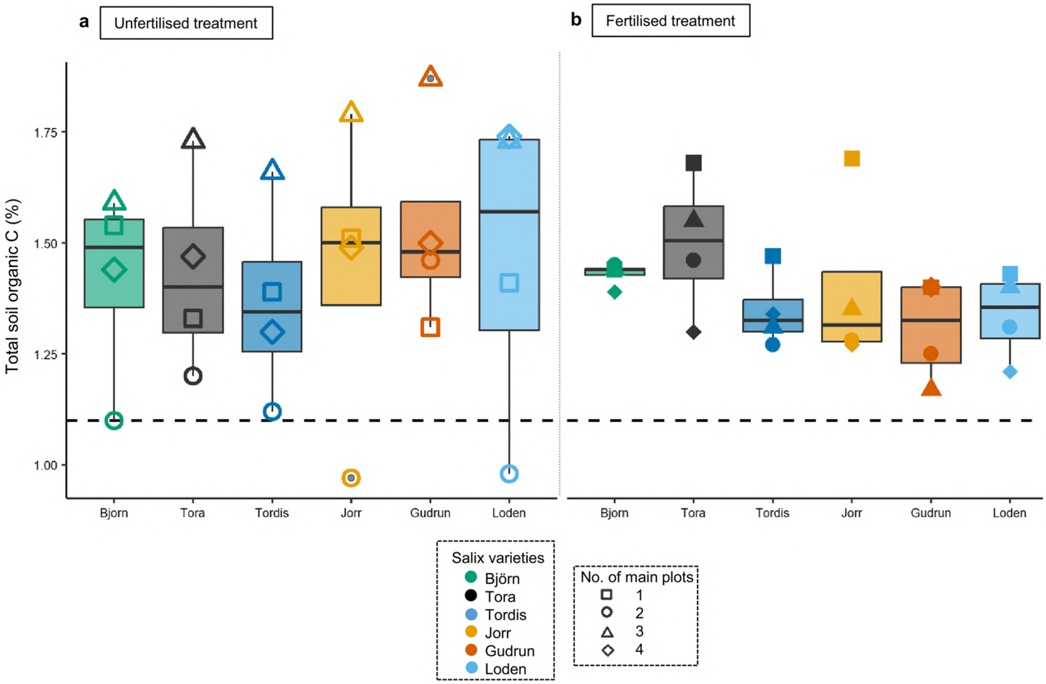


**Fig. 2** Total soil organic C (%) in samples from different willow monoclonal subplots: (**a**) the four unfertilised and (**b**) the four fertilised main plots. Boxplots show (i) the median as crossbars inside boxes, (ii) 75th and 25th percentile as crossbars on the top and the bottom of boxes respectively, (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

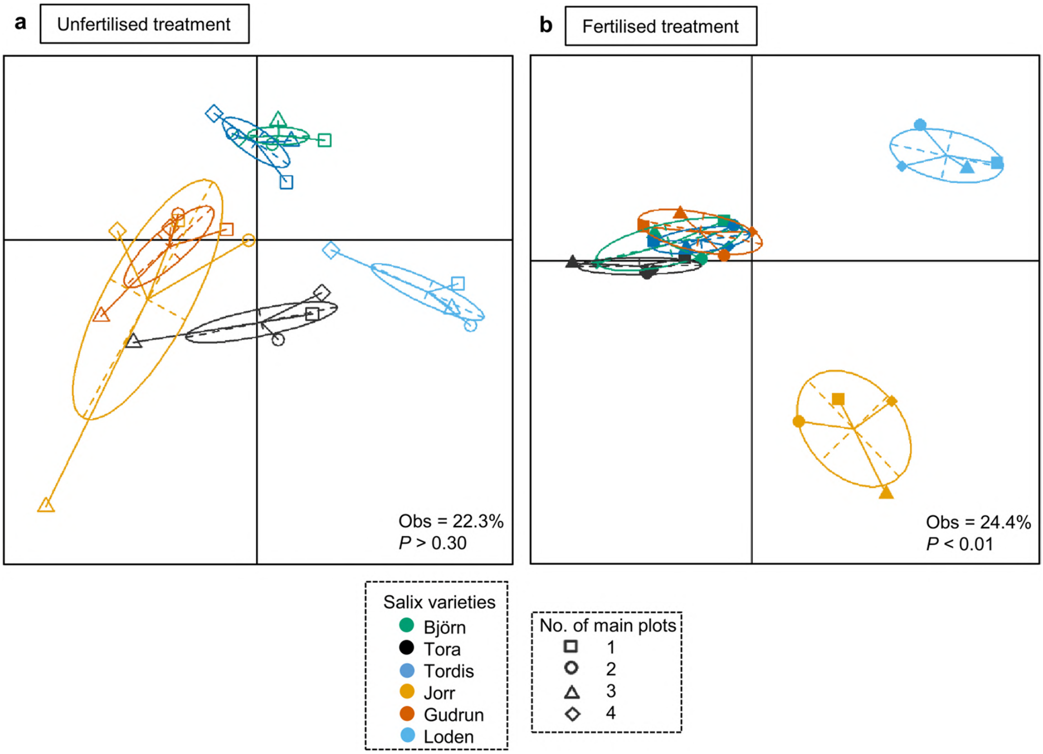


**Fig. 3** Between-class analysis (BCA) of the full mid-infrared spectra of the soil from beneath the different *Salix* varieties: (**a**) unfertilised treatment and (**b**) fertilised treatment. The abbreviation "Obs" refers to the percentage of the overall inertia in the data explained by the variable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

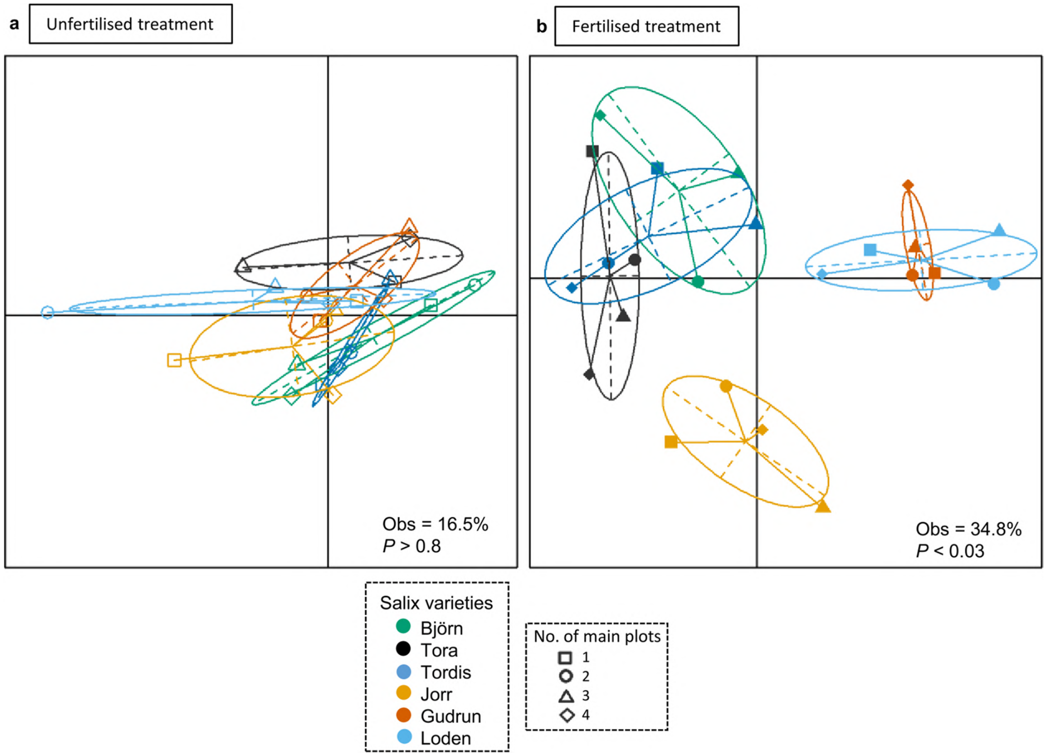


**Fig. 4** Between-class analysis (BCA) of identified pyrolysis products of soil organic matter among different willow monoclonal subplots for (**a**) 23 soil samples in the unfertilised treatment (one NA value for Tora main plot 2) and (**b**) 23 soil samples in the fertilised treatment (one NA value 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<br>% | 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

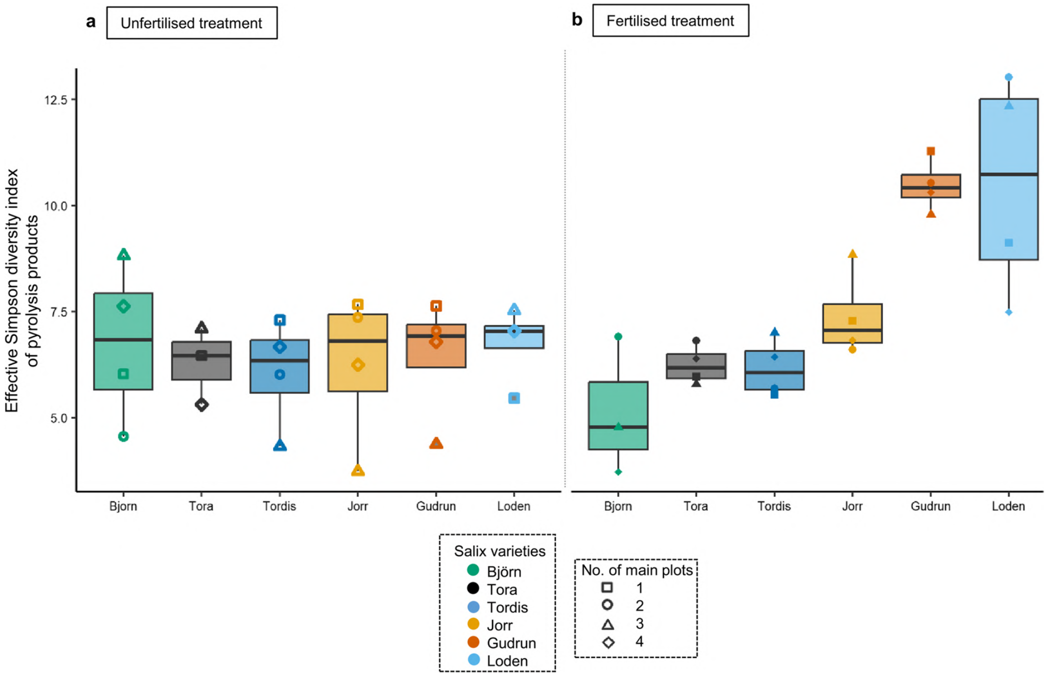


**Fig. 5** Effective Simpson diversity index of identified pyrolysis products of soil organic matter from different willow monoclonal subplots: (**a**) unfertilised treatment and (**b**) the fertilised treatment. Boxplots show (i) the median as crossbars inside boxes, (ii) 75th and 25th percentile as 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.

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**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.

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**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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# Supplementary Materials for

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

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**This file includes:**

Fig. S1. Between-class analysis (BCA) carried out on the entire mid-infrared spectra of the soil from beneath the different Salix varieties in the fertilised treatment.
Fig. S2. Between-class analysis (BCA) carried out on the entire mid-infrared spectra of the soil from beneath the different Salix varieties in the control treatment.
Fig. S3. Between-class analysis (BCA) of identified pyrolysis products of soil organic matter among different *Salix* varieties in fertilised treatment.

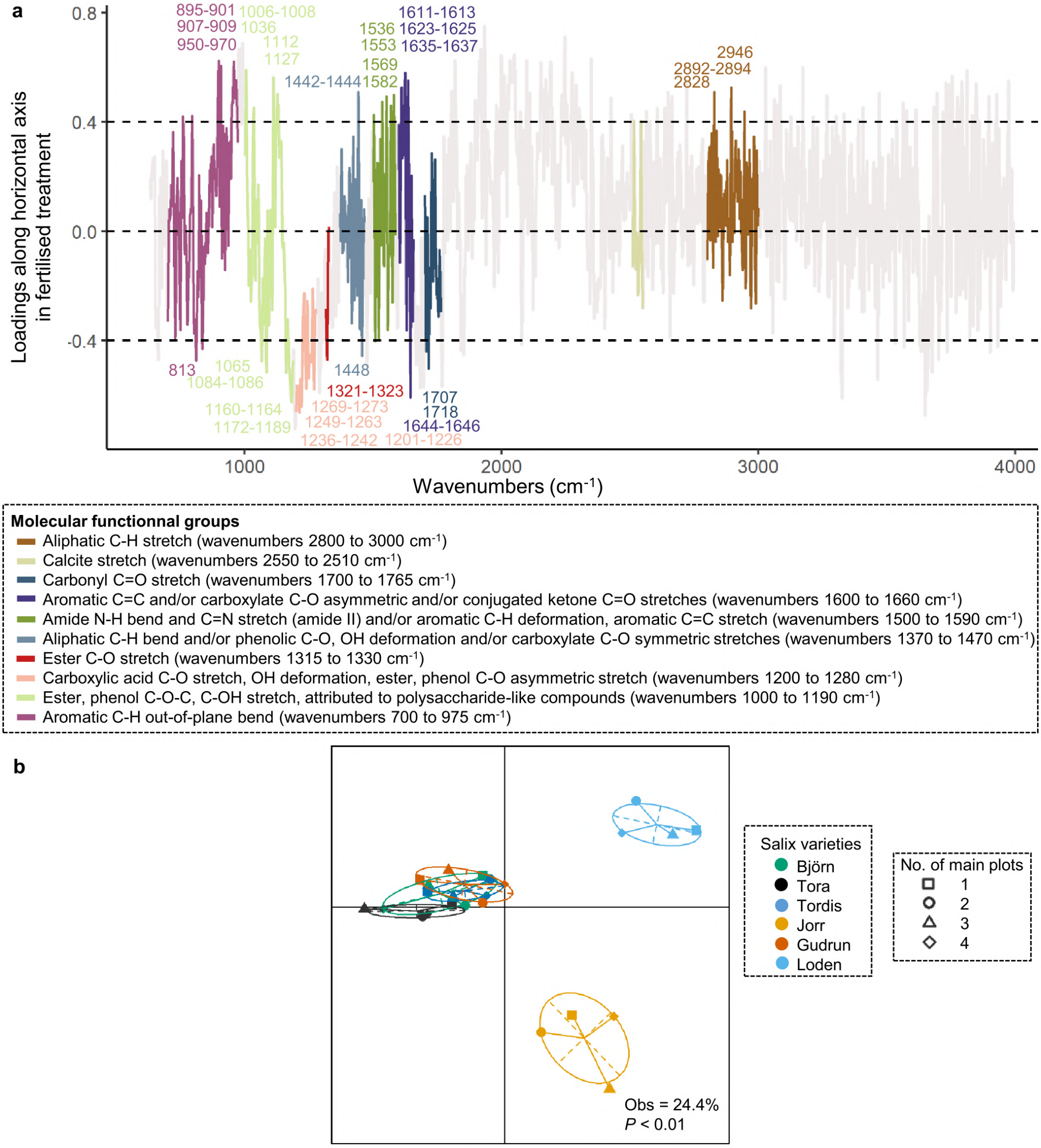

**Fig. S1.** Between-class analysis (BCA) carried out on the entire mid-infrared spectra of the soil from beneath the different Salix varieties in the fertilised treatment. **(a)** The loadings of wavenumbers (cm$^{-1}$) along the horizontal axis where the projected unconstrained inertia of the principal component analysis explained by the variable "Salix varieties" was 9%. **(b)** The graph of individuals. The wavenumbers associated with the gray line were not mentioned in the results sections as they were dominantly influenced by minerals or had a lower weight to discriminate varieties.

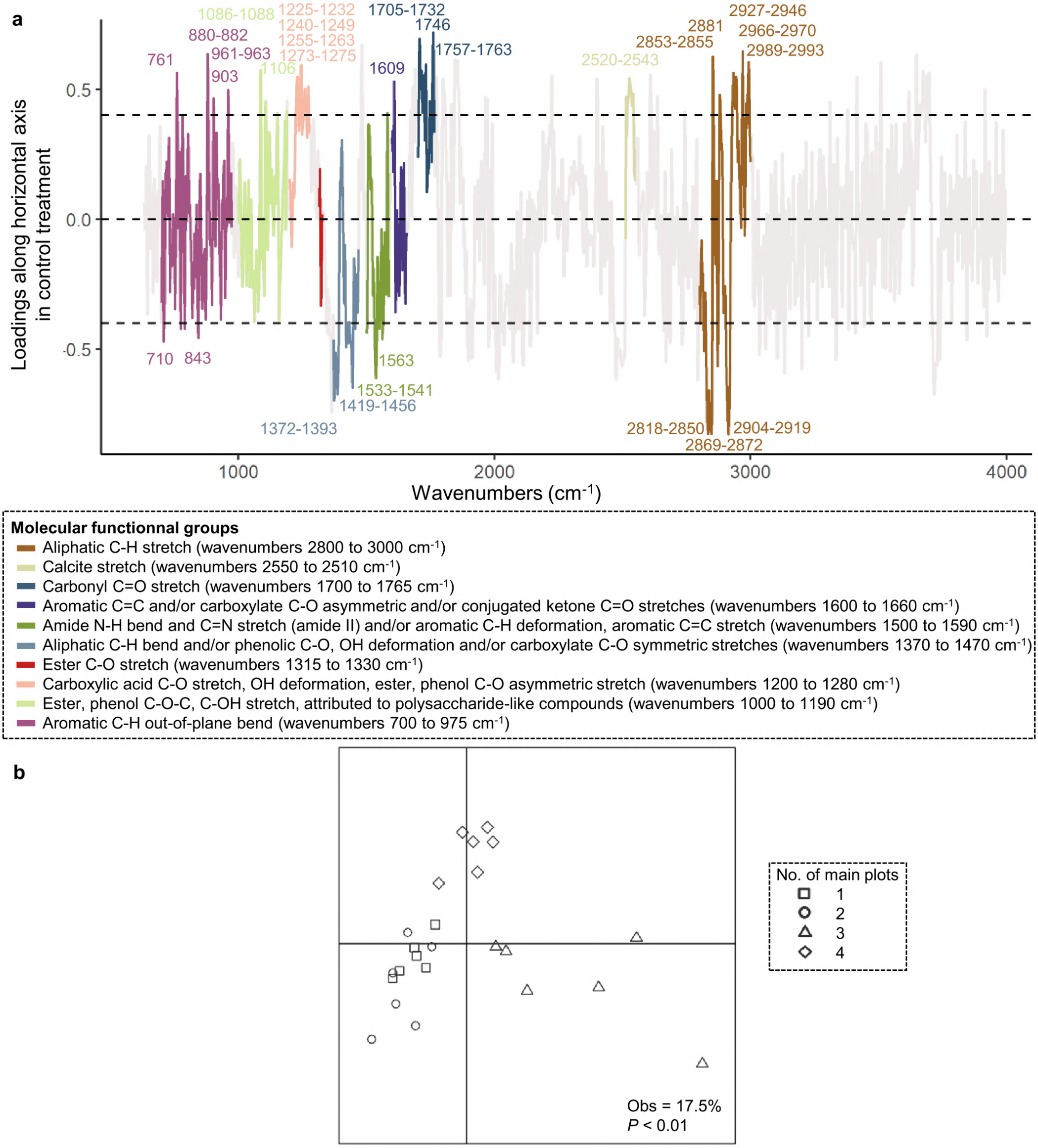

**Fig. S2.** Between-class analysis (BCA) carried out on the entire mid-infrared spectra of the soil from beneath the different Salix varieties in the control treatment. **(a)** The loadings of wavenumbers (cm$^{-1}$) along the horizontal axis where the projected unconstrained inertia of the principal component analysis explained by the variable "No. of main plots" was 8.7%. **(b)** The graph of individuals. The wavenumbers associated with the gray line were not mentioned in the results sections as they were dominantly influenced by minerals or had a lower weight to discriminate varieties.

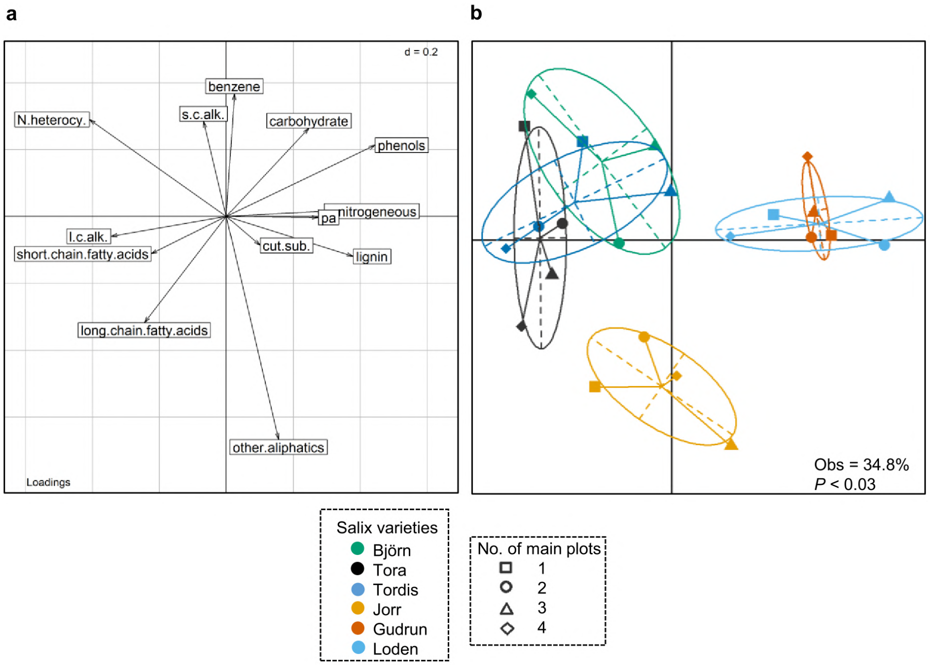

**Fig. S3.** Between-class analysis (BCA) of identified pyrolysis products of soil organic matter among different *Salix* varieties in fertilised treatment. **(a)** The loadings of biochemical categories. **(b)** The graph of individuals. Abbreviations of biochemical categories: lignin that originated from higher plant (**lignin**), long chain fatty acids that originated from higher plant (**long.chain.fatty.acids**), short chain fatty acids that originated from microbial community (**short.chain.fatty.acids**), nitrogeneous compounds of multiple origins (**nitrogeneeous**), carbohydrates of multiple origins (**carbohydrate**), other aliphatics of multiple origins (**other.liphatics**), phenols of multiple origins (**phenols**), benzene derivatives of multiple origins (**benzene**), N-heterocyclic compounds of multiple origins (**N.heterocyclic**), polyaromatic compounds of multiple origines (**pa**), cutin and/or suberine originated compounds from higher plant origin (**cut.sub.**), short chain alkane from microbial origin (**s.c.alk.**), long chain alkane from higher plant origin (**l.c.alk.**).

# Carbon dynamics in soils – Does the diversity of organic inputs matter? A bioenergetics approach

Louis J.P. Dufour

The public defence for the degree of Doctor of Philosophy (PhD) will take place in Sal L Undervisningshuset, Ultuna on 26th of May, 2025, at 13:00.

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Soil microorganisms require energy for their maintenance, and they acquire it from the decomposition of organic matter. This thesis investigated how diversified organic inputs affect the turnover of organic matter in soils using a microbial bioenergetics approach. Molecular richness of organic inputs may be important in carbon limited environments, but soil carbon cycling is best understood through the lens of interactions between organic matter properties and activity of the microbial community in soils.

**Louis J.P. Dufour** received his PhD education at the Department of Soil and Environment, SLU Uppsala, Sweden. He holds a Master of Science (MSc) in Biology of Microorganisms from Strasbourg University and an Engineering Degree in Food, Agronomic and Environmental Sciences from VetAgroSup, Clermont-Ferrand, France.

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