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

Louis J.P. Dufour [a,*], Anke M. Herrmann [a], Julie Leloup [b], Cédric Przybylski [c], Ludovic Foti [b], Luc Abbadie [b], Naoise Nunan [a,b]

[a] Department of Soil and Environment, Swedish University of Agricultural Sciences, P.O. Box 7014, SE-75007, Uppsala, Sweden
[b] Institute of Ecology and Environmental Sciences, iEES Paris, Sorbonne Université, CNRS, IRD, INRA, UPEC, Univ Paris Diderot, 4 place Jussieu, 75005, Paris, France
[c] Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, IPCM, 4 place Jussieu, 75005, Paris, France

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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 (ΔE) and the weighted average standard state Gibbs energies of oxidation half reactions of organic C (ΔG°_{Cox}). ΔE was measured as the heat of combustion using bomb calorimetry. ΔG°_{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

Abbreviations: ROI, Energetic return on investment; ΔE, Heat of combustion; NOSC, Nominal oxidation state of carbon; ΔG°_{Cox}, Standard state Gibbs energies of oxidation half reactions of organic carbon.

* Corresponding author.
E-mail addresses: Louis.Dufour@slu.se (L.J.P. Dufour), Anke.Herrmann@slu.se (A.M. Herrmann), Julie.Leloup.1@sorbonne-universite.fr (J. Leloup), Cedric.Przybylski@sorbonne-universite.fr (C. Przybylski), Ludovic.Foti@gmail.com (L. Foti), Luc.Abbadie@sorbonne-universite.fr (L. Abbadie), Naoise.Nunan@cnrs.fr (N. Nunan).

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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 (ΔG°_{Cox}) (LaRowe and Van Cappellen, 2011). The ΔG°_{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 (ΔE) and the ΔG°_{Cox} of the molecular species contained in the organic matter. We estimated the ΔG°_{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 ΔE 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

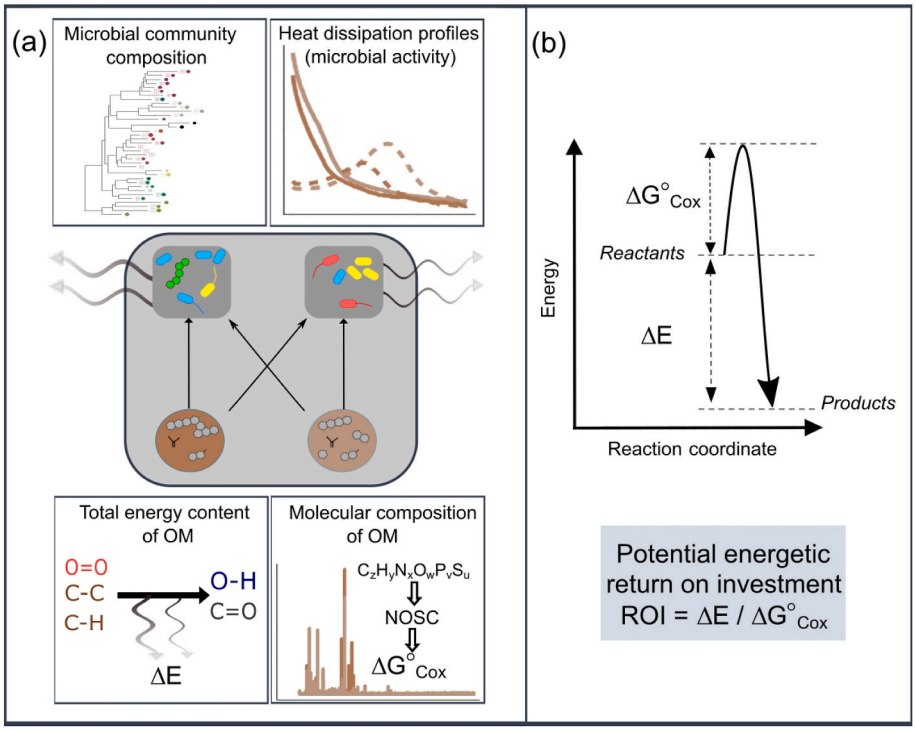

Fig. 1. Conceptual representation of the experimental design. (a) Six soil organic matter solutions containing different molecular profiles but the same quantity of organic C were added cross-wise to 6 soils (central panel). The soils harboured distinct bacterial communities (top left panel). The metabolic 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 ΔG°_{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 ΔG°_{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 ΔG°_{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 (Δmc = 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).

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

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:

$$\text{Sum of the intensity weighted NOSC} = \Sigma\,(NOSC \times RI_{NOSC}) \quad (2)$$

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 (ΔG°_{Cox}) (LaRowe and Van Cappellen, 2011). As the ΔG°_{Cox} of each molecular formula is additive, the bulk ΔG°_{Cox} of each soluble organic matter was calculated in J $mmol^{-1}$ of C at 25 °C, 100 kPa as follows:

$$\Delta G^\circ_{Cox} = 60.3–28.5 \times \text{Sum of the intensity weighted NOSC} \quad (3)$$

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:

$$ROI = \Delta E / \Delta G^\circ_{Cox} \quad (4)$$

where ΔE is the total net energy available (determined by bomb calorimetry) and ΔG°_{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 ΔG°_{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

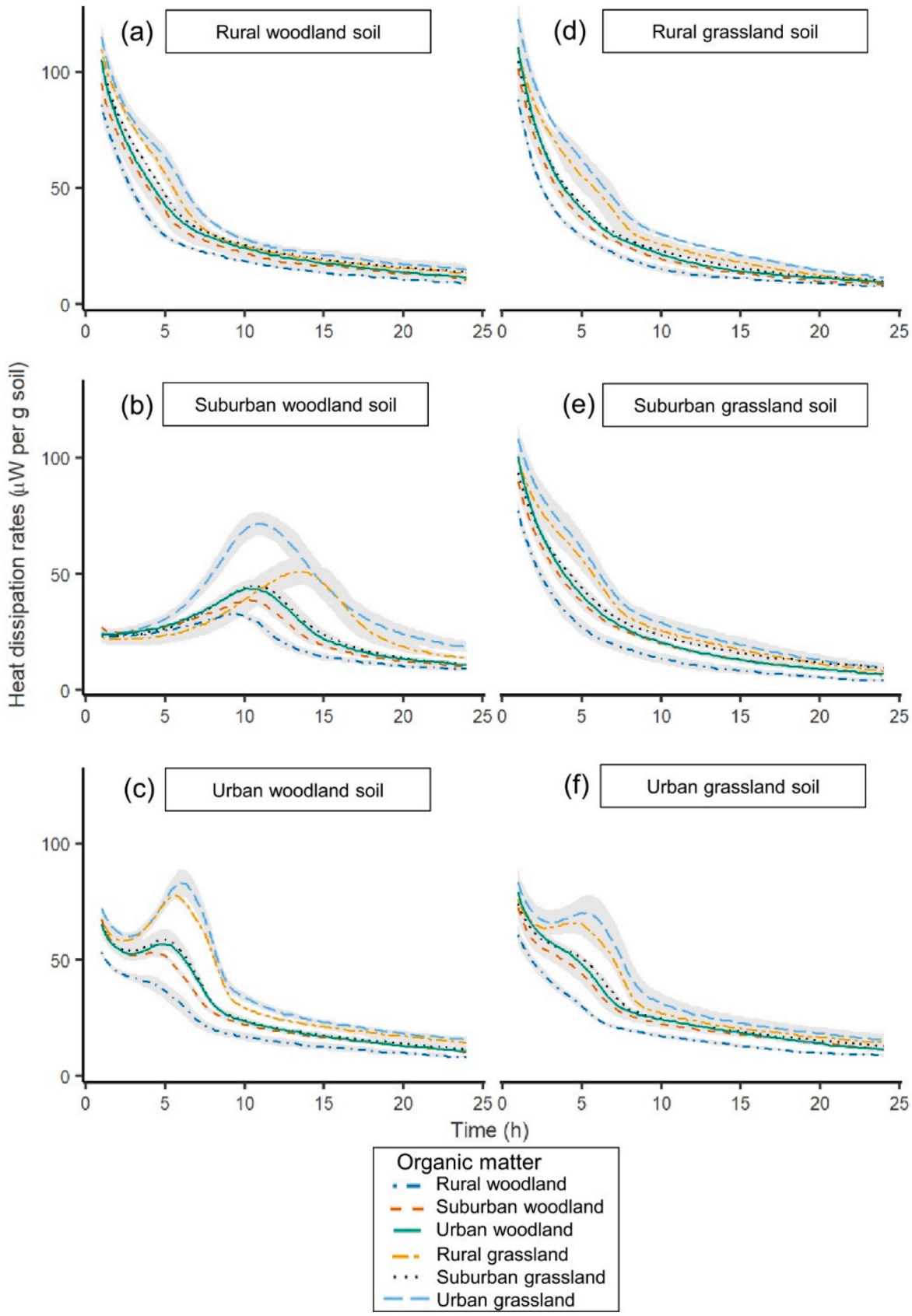

Fig. 2. Heat dissipation patterns from soils after the addition of 0.3 mg soil organic carbon. **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soils. Each curve depicts the mean (n = 4) heat dissipation 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

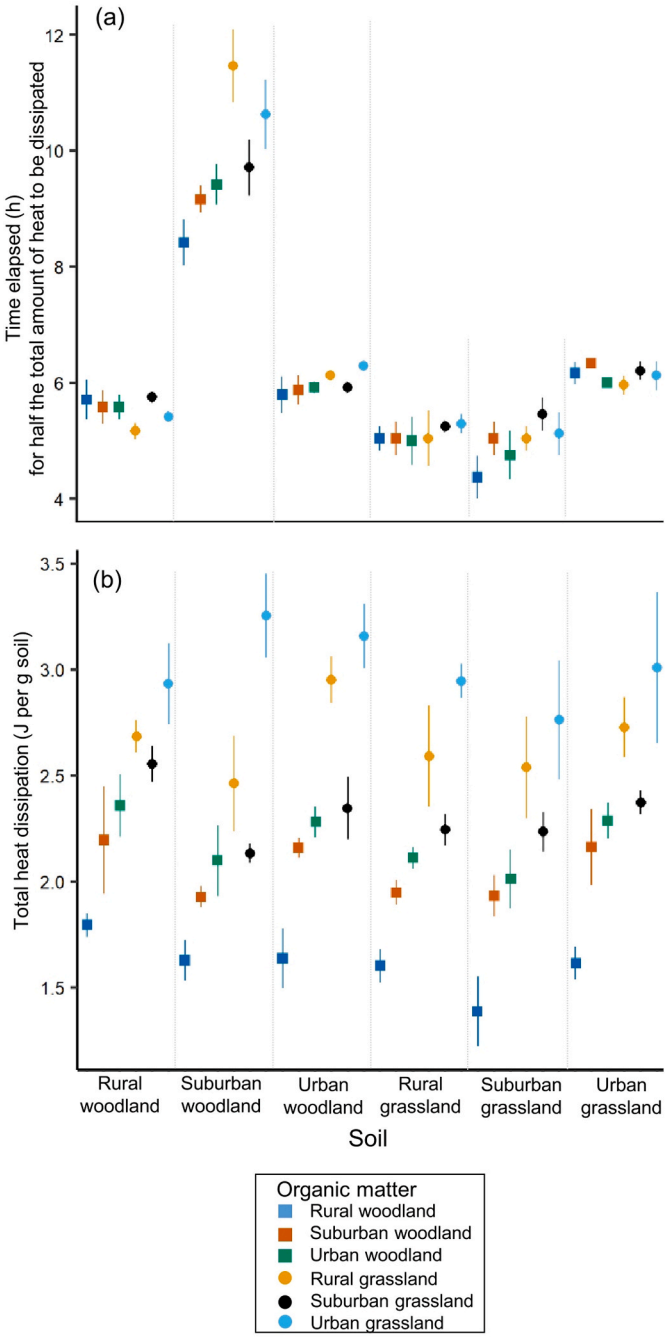

Fig. 3. Variables describing the heat dissipation curves. **(a)** Time elapsed for half the total amount of heat to be dissipated, and **(b)** total heat dissipation. Each symbol depicts the mean ± one standard deviation (n = 4) after subtraction of the mean heat dissipation in control soils that only 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	4.9705×10^{-13}
		interaction OM: Soil	25	F = 6.3925	3.2681×10^{-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	1.29×10^{-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

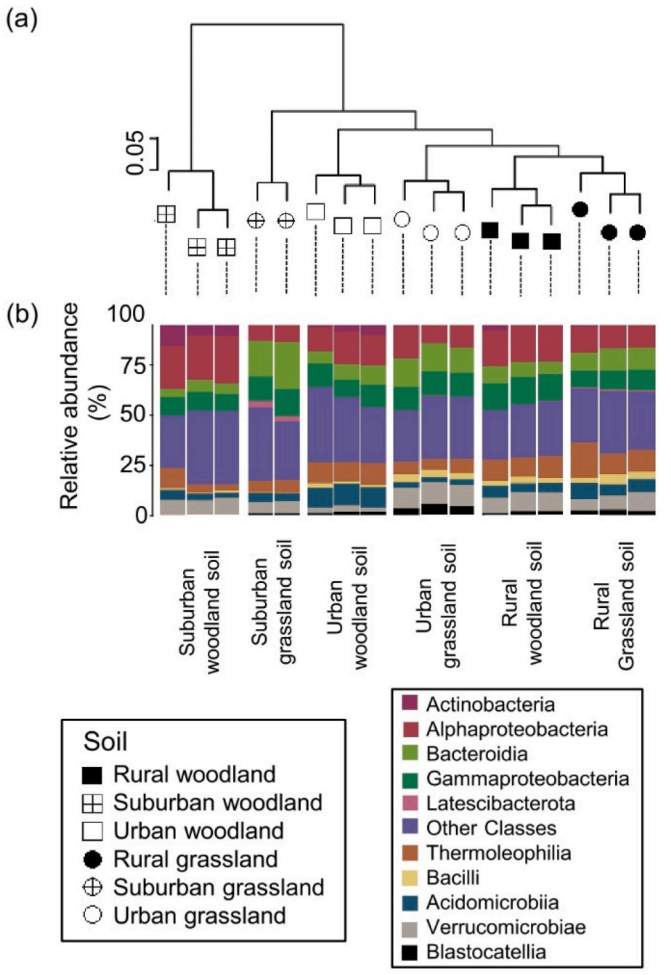

Fig. 4. Differences in bacterial community composition between soils. **(a)** Hierarchical clustering of weighted UniFrac distances of rarefied ASVs data using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), and **(b)** taxonomic profiles (at the class level) labeled by soil were ordered 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 (ΔG°_{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	ΔG°_{Cox} (J $mmol^{-1}$ of C)	ROI (ΔE/ ΔG°_{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.

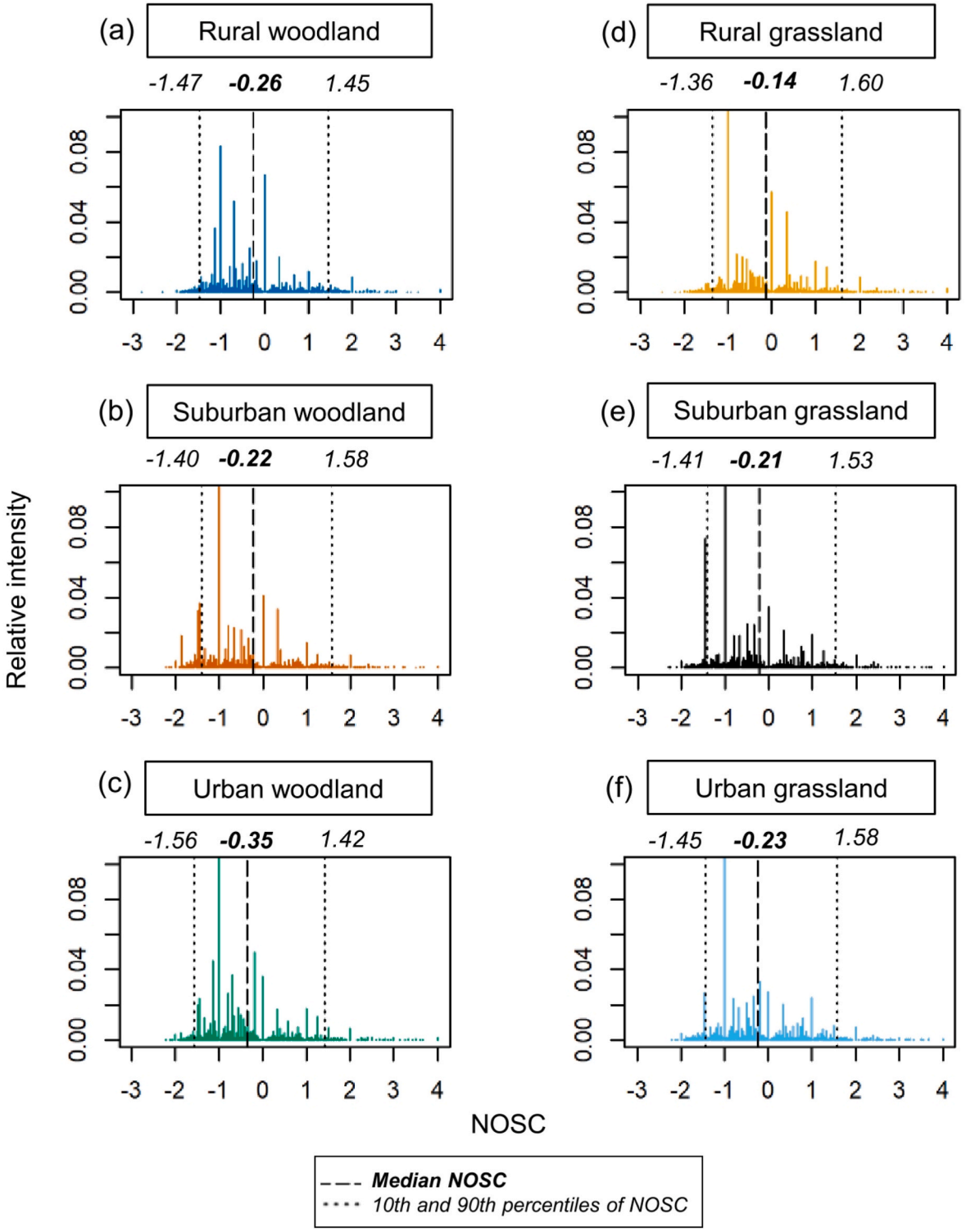

Fig. 5. Distribution of the relative intensities of nominal oxidation state of carbon (NOSC) in water-soluble organic matter (OM). **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soluble OM. A 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 ΔG°_{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

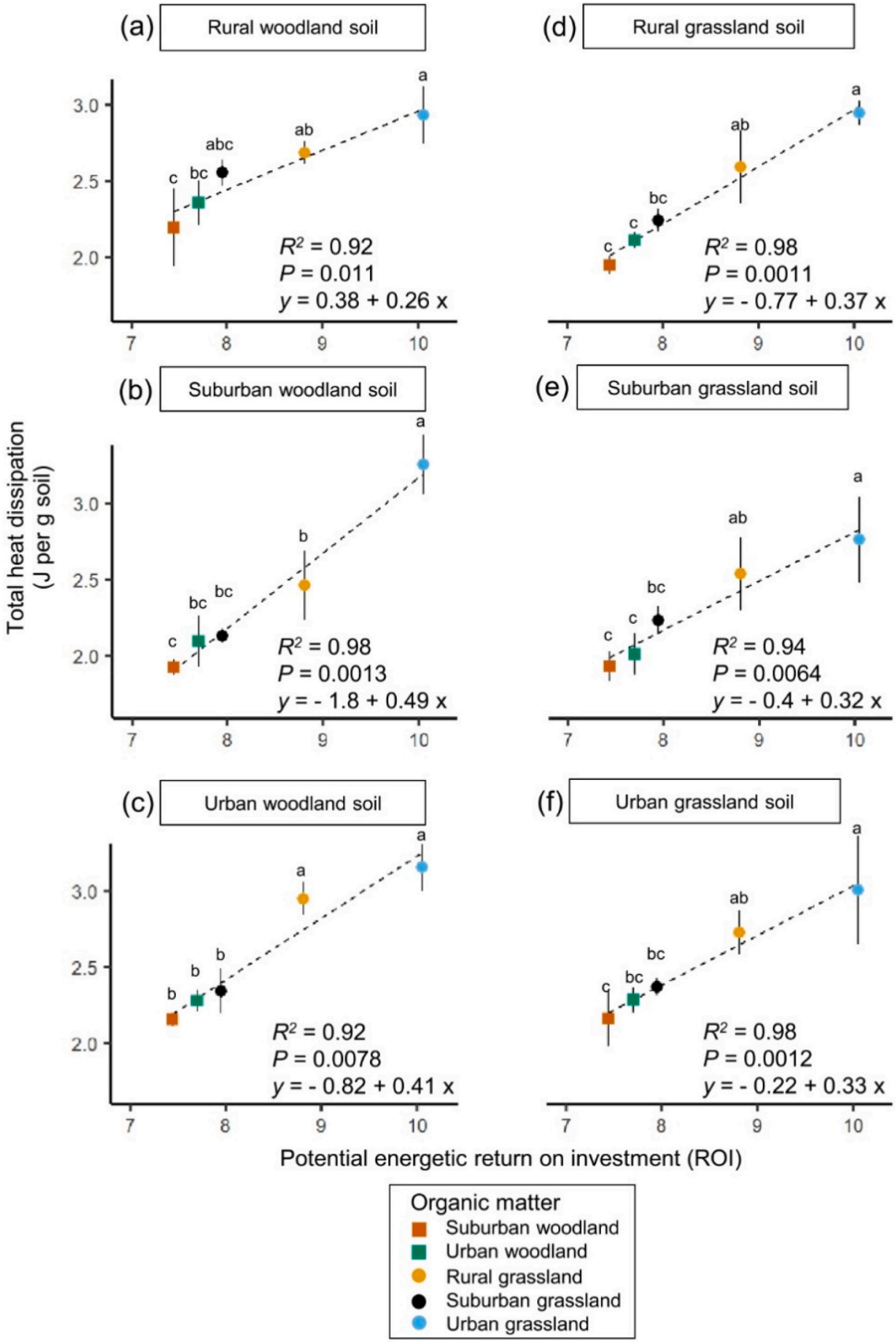

Fig. 6. Relationships between the total heat dissipation and the potential energetic return on investment of water-soluble organic matter. **(a)** Rural woodland, **(b)** suburban woodland, **(c)** urban woodland, **(d)** rural grassland, **(e)** suburban grassland, and **(f)** urban grassland soils. 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 (ΔE) 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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