Energetic return on investment determines overall soil microbial activity.

Louis Dufour (louis.dufour@slu.se)
Swedish University of Agricultural Sciences https://orcid.org/0000-0002-8569-6304
Anke Herrmann
Swedish University of Agricultural Sciences
Julie Leloup
Institute of Ecology and Environmental Sciences, iEES Paris, Sorbonne Universitié, CNRS, IRD, INRA, UPEC, Univ Paris Diderot
Cédric Przybylski
Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, IPCM
Ludovic Foti
Institute of Ecology and Environmental Sciences, iEES Paris, Sorbonne Universitié, CNRS, IRD, INRA, UPEC, Univ Paris Diderot
Luc Abbadie
Institute of Ecology and Environmental Sciences Paris
Naoise Nunan
Centre national de la Recherche Scientifique https://orcid.org/0000-0003-3448-7171

Article

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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. We suggest that a full understanding of microbial communities' involvement in soil C dynamics can only be attained when the interaction between the properties of both available organic C and microbial communities are accounted for. Here, we show that the potential energetic return on investment, derived from an energetic analysis of available organic C, is strongly related to the overall metabolic response of microbial decomposers. We further show that microbial communities do not all obtain the same energetic return on investment when metabolising the same organic C, suggesting that the response also depends on the intrinsic properties of the microbial communities.

Introduction

One of the principal sources of uncertainty in atmospheric CO_2 projections in earth system models is associated with the response of the land carbon (C) cycle to changes in environmental conditions[1]. The uncertainty is due to the fact that we still do not have a sufficiently fine understanding of the drivers and constraints that regulate C dynamics in soil. There is a general consensus that microbial access to substrate, organic matter association with mineral surfaces and anaerobic conditions all contribute to organic C persistence in soil[2, 3, 4]. The hierarchy of involvement of these mechanisms in regulating organic C persistence is also believed to change as a function of soil physicochemical properties[5]. However, whilst these mechanisms can explain why organic C remains in soil over the longer term, they are less useful for understanding decomposition rates in microbial hotspots, which have been estimated to account for the majority of overall CO_2 emissions from soils[6].

Microbial hotspots are defined as sites with significantly higher microbial activity than the surrounding bulk soil. In these hotspots, examples of which are the rhizosphere or the detritusphere, abiotic constraints on microbial activity are lower due to the regular and non-limiting supply of organic substrate. In such situations, where the abiotic constraints on microbial activity are negligible, 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. The processing of substrate C then depends on the type of metabolism and the energetic requirements of the cellular processes present[7, 8] and 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[9]. Recent evidence also suggests that the taxonomic composition and diversity of the microbial communities can also affect how substrate C is processed[10, 11], presumably a consequence of lower metabolic costs associated with resource acquisition within the communities, due to cross-feeding for example.

The type of organic C being consumed also has some bearing on how it is processed. It has long been established that organic substrates stimulate microbial activity to a greater or lesser extent, depending on their nature[12, 13]. 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[14, 15]. However, the organic matter that is available to decomposers in soil displays a high degree of molecular heterogeneity[16] and the effects of heterogeneous substrate on microbial activity is, as yet, unclear[15]. Microbial communities acquire resources from outside the cell and, in doing so, incur a metabolic cost associated with the production of extracellular enzymes and membrane transport proteins[17]. 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[18].

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[19]. Whilst this is conceptually appealing, there is no empirical evidence of microbial energetic return on investment being related to the decomposition of organic substrate. Theoretically, the energetic return on investment can be estimated using empirical thermodynamic, kinetic and physiological data in metabolic network models[20, 21]. However, the empirical data required to parameterise such models are not available for the large diversity of organic compounds[22], enzymes[23] and microorganisms found in soil. Here, we propose an experimental approach to estimate the energetic return on investment that microbial decomposers can acquire from the consumption of organic matter. This metric is determined as the ratio between the energy available in the organic matter and the decomposition activation energy of each molecular species contained in the organic matter. The energy available in the organic matter was measured as the heat of combustion with bomb calorimetry[24] and the activation energy was estimated using the nominal oxidation state of carbon (NOSC) of each of the molecular species[25].

The objective of the study was to determine how properties of the organic matter (composition, molecular or energetic heterogeneity) and of the resident soil microbial communities (composition, diversity) affect the metabolic response of microbial communities. The relationship between the energetic return on investment and microbial activity was determined by cross amending six soils with soluble organic matter from the same six soils and measuring the heat dissipated due to the increase in microbial metabolic activity (Fig. 1). Hence, the decomposition of readily available organic matter amendments was examined. The constraints on microbial activity associated with the physical or mineral protection of organic matter were removed by adding a sufficiently large amount of soluble organic substrate to ensure that all microbial decomposers were in the conditions that one would expect to find in activity hotspots. Specifically, the heat dissipation curves were compared with the organic matter and microbial properties mentioned above. 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 NOSC of the molecular species. The organic matter heterogeneity was estimated as the diversity of molecular compounds and as the diversity of energy

forms present in the organic matter. In order to determine how different microbial decomposers 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), as there is a very general relationship between taxonomic and functional profiles in microbial communities[26].

Our hypotheses were twofold: i) the molecular heterogeneity of the added organic matter is negatively related to microbial metabolism, due to the higher cost involved in metabolising more heterogeneous organic matter; ii) the greater the potential energetic return on investment available to microbial decomposers in organic matter, the greater the metabolic response. We show that there is a significant positive relationship between the energetic return on investment that is potentially available to microorganisms and the overall metabolic heat response of microbial decomposers in soil. We further show that different microbial communities do not exploit the energetic return on investment in the same manner, resulting in different metabolic temporal dynamics.

Results And Discussion

Temporal and hierarchical pattern of microbial activity in soils. The dynamics of the microbial metabolic response to additions of heterogeneous organic matter are shown in Fig. 2. There were a variety of responses, including monotonic decreases from an initial high heat dissipation rate, hump shaped profiles or profiles characterised by an increase in heat dissipation after an initial decrease. There were clear differences among soils and organic matters. The shape of the curves (i.e. the metabolic dynamics) tended to depend on the soil and there was a hierarchy among the organic matters, with heat dissipation generally being highest when soils received urban or rural grassland organic matter and lowest when soils received rural or semi-urban forest 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). The time elapsed for half the total amount of heat to be dissipated (Fig. 3a) showed significant differences among soils ($P < 0.001$). Soils were associated either with an early heat dissipation phase or with a later heat dissipation phase. The semi-urban forest soil showed a particularly late heat dissipation phase. The differences in dynamics were also dependent on the organic matter added, but to a lesser extent ($P < 0.001$), and there was an interaction between soils and organic matter ($P < 0.001$), suggesting that the differences among soils were not constant across organic matters (Table 1a). Although, there were differences in the dynamics of heat dissipation, no differences in the overall microbial activity were apparent between soils (Fig. 3b). There was, however, a significant hierarchy related to the origin of the organic matter added within each soil. The hierarchy was completely consistent across soils, with the urban grassland organic matter always resulting in higher overall heat dissipation ($P < 0.001$) and the rural forest organic matter producing the lowest dissipation of heat ($P < 0.001$) (Figs. 2 and 3). This suggests that the composition of the organic matter affected the overall metabolic response, regardless of the properties of the soils. There was a soil x organic matter interaction ($P < 0.01$), indicating that,

although the organic matter hierarchy was consistent, the differences among organic matter types were not constant across soils (Table 1b).

We then determined the extent of the relationship between the heat dissipation dynamics either with the composition of soil bacterial communities or with the composition of the added organic matter, using Mantel tests. These showed that microbial community composition was mostly significantly related to the dynamics of heat dissipation, but that the composition of the organic matter did not have any significant impact (Table 2). The semi-urban forest soil not only had the slowest heat dissipation dynamics, but also showed the most divergent bacterial community composition (Fig. 4a) and the lowest diversity index (Fig. 4b; Supplementary Table 1). However, there were no significant relationships between the time elapsed for half the total amount of heat to be dissipated and the effective Simpson diversity index of the bacterial communities, regardless of the organic matter added to the soils (Supplementary Fig. 1). In the past, there has been much debate in the literature about whether microbial use of organic substrate is controlled by the intrinsic properties of the microbial communities or by the properties of the organic substrate supply: a number of contributions[27, 28] have argued that C dynamics in soil can be understood on the basis of composition of organic substrate available for microbial use; the implication being that the differences in the functioning of distinct microbial communities is negligible relative to the differences due to the soil organic substrate. Recent studies have claimed that microbial physiology affects the outcome of microbial use of organic substrate[29, 30]. Strickland et al.[31] and Nunan et al.[32] have also shown that decomposer use of organic matter is dependent on the composition of decomposer communities, in situations where organic matter availability is not limiting. Similarly, Domeignoz-Horta et al.[11] observed a relationship between soil bacterial community growth rates and diversities in moist but not in dry conditions, where one might expect abiotic constraints to be greater. The data presented here suggest that the dynamics of organic matter consumption is mostly related to the taxonomic composition rather than the diversity of the microbial communities (Table 2 and Supplementary Fig. 1), at least in the case of short-term dynamics, where abiotic constraints are reduced. Furthermore, the differences in metabolic dynamics displayed by the microbial communities suggests the presence of different metabolic pathway profiles at the community level. The differences may have arisen due to different selective pressures to which the communities are subjected. For example, microbial communities that have metabolic pathways associated with lower enzyme demand can allocate free energy to other cellular processes that contribute to higher microbial activity rates[33, 34].

We analysed the molecular composition and diversity of the organic matter using ultra high resolution mass spectrometry (FT-ICR-MS) and deduced the composition and diversity of the energy forms (i.e. the NOSC) from the elemental composition of the molecular species present in the organic matter[25]. There were compositional differences among the organic matters. All of the organic matters were composed of the same compound classes in roughly the same proportions (supplementary Fig. 2), but the compositional profiles (Supplementary Fig. 3) and diversities of molecular formulae (Table 3) were different. This translated into different energetic profiles (Fig. 5) and diversities of energetic forms (Table 3). None of these metrics used to describe the organic matter were related to the heat dissipation

dynamics nor to the overall heat dissipation, leading us to reject our first hypothesis. However, in some soils, the overall energy contained in the different organic matters, estimated as the heat of combustion by bomb calorimetry[24], and the intensity weighted averages of the N:C ratio of organic matter were significantly related to the total amount of heat dissipated during the incubations (Supplementary Figs. 4–5). These data suggest that neither the composition of the organic matter, the overall energy availability, nor the diversity of the forms of available energy, determine directly the overall metabolic response of microbial communities when consuming organic matter. This may be viewed as a surprising conclusion to arrive at, as the oxidation of molecular species with higher NOSC is more favourable from a thermodynamic point of view[25]. However, microbial communities have to make metabolic investments (e.g. production of enzymes and transport proteins) in order to acquire resources[35, 36] and the magnitude of these investments depends on the composition of the organic matter that is available[37, 38]. 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 form of the energy available.

Energy return on investment (ROI) of soluble organic matters. We therefore estimated the energetic return on investment that is potentially available to microbial decomposers when decomposing the different organic matters. This was done using the bomb calorimetry data as an estimate of the total net energy available in the substrate[24] and the NOSC data as an estimate of the activation energy required to acquire energy from the available molecular species[39]. The rationale behind this approach is based on the fact that molecular species containing carbon atoms that are more reduced on average (i.e. have a lower average NOSC) tend to have higher oxidation activation energies[25, 40, 41] (i.e. higher Gibbs energies for the oxidation half reaction of organic compounds (ΔG°_{Cox})). As enzymes catalyse reactions by decreasing the energy of activation, we make the assumption that microbial decomposers incur higher metabolic costs when acquiring energy through reactions with higher energies of activation (Fig. 1). The higher microbial metabolic costs may be due to the need to produce enzymes in greater quantity[42], to make use of additional co-factors[41] or to produce larger and more costly enzymes[43]. It has been shown, for example, that hydrolases use a variety of strategies, including the use of co-factors and different enzyme sizes, to ensure consistent enzyme efficiencies across a wide range of substrate types[43].

There were strong, significant positive relationships between the energetic return on investment that soil microorganisms can make when processing the organic matter and the overall heat dissipation (Fig. 6). The idea that the energetic return on investment plays a significant role in the dynamics of C in soil has been proposed on repeated occasions in the literature[44, 45]. However, empirical evidence has been rather thin on the ground. The analysis proposed here not only provides evidence that the overall metabolic response of microbial communities is related to the energetic return on investment of the heterogeneous organic substances, it also shows that not all microbial communities make use of the potential return on investment in the same way. The regression coefficients describing the relationships between the potential energetic return on investment and total heat dissipation differed among soils, suggesting that

the community composition influences the actual energy return on investment that microbial decomposers can make when processing available organic matter. Relationships between the potential return on investment and total heat dissipation with steeper slopes are indicative of metabolisms that are more sensitive to changes in organic substrate. This was the case for the microbial communities in the semi-urban and urban forest soils and in the rural grassland soil. Such metabolisms, may at the scale of individual taxon, be the result of a smaller genome that constrains the range of organic substrates that can be processed[10], but it is not clear whether this remains true at the community scale. It is interesting to note that the three microbial communities with the steepest slopes (Fig. 6) did not have similar taxonomic compositions nor diversity indices (Fig. 4; Supplementary Fig. 1), suggesting that this relationship should be investigated further. It may be due to the fact that fungal and protist communities were not included in the analysis[46].

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. We therefore propose that the energetic return on investment decomposers can achieve when consuming organic matter is a better proxy for predicting microbial activity than the molecular diversity of organic matter alone[47]. It has recently been suggested that the persistence of C in soil can be understood as the outcome of interactions between the molecular heterogeneity of organic matter and spatial and temporal heterogeneities which reduce the potential return on investment that microbial decomposers can obtain from degrading the organic matter[48]. This concept is not reflected in the majority of soil C dynamics models, which take the view that the dynamics are either related to the properties of the organic C in soil (the size of C pools[49]) or related to the ecophysiology of the microbial decomposers[50]. The metabolic cost associated with the consumption of different types of organic substrate is implicitly represented in the continuum of C qualities model[51]. 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[21]. 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 biotic and abiotic changes on soil C dynamics. Of course, the relationship found here would have to be tested over a wider range of soils, land-use managements and climates.

In a wider perspective, the experimental approach proposed in this study might be useful in determining how different plants can affect/stimulate microbial activity through molecular and energetic analysis of the rhizodeposits. It has been suggested that microbial processing of rhizodeposited C leads to more persistent C in soil[52, 53], and that processing by different microbial communities leads to different quantities of mineral associated organic matter[9]. The different metabolic dynamics shown in the present study lends credence to the suggestion that different microbial communities process organic matter differently. The proposed framework may therefore further our understanding of the role of microbial processing of rhizodeposited C contributing to its persistence in soil[54].

Methods

Soil and water extractable organic matter. Soils were sampled in June 2016 from six sites (Supplementary Table 2), associated with two land-use types (forest or grassland), along an urban pressure gradient (rural, semi-urban and urban areas) [55]. The soils were chosen to ensure that a range of soluble organic matter and microbial community compositions were obtained. Three subsamples were taken from the top-surface (10 cm) after removal of the litter layer. The inter-subsample distance was at least 5 meters. The soil was then sieved (< 2 mm), air dried, mixed and stored at 4°C prior to initiating the experiment.

Water extractable organic matter was extracted in triplicate by shaking soil samples with H_2OmQ (1:10 soil:water) at 60°C for 30 minutes and subsequently centrifuging the soil suspension (5250 × g) for 10 min at 4°C [56]. The supernatant was filtered through glass fibre 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 organic C and total N content of the water extractable organic matter and of the soils were determined using an elemental analyser that had been calibrated with tyrosine (Supplementary Tables 2–9). Prior to analysis, the inorganic carbon of the water extractable organic matter was removed by acid fumigation[57].

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 matters, we cross amended the six soils with water extractable 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 quatruplicate, making a total of 168 samples (6 soils × 7 treatments × 4 replicates). They were analysed in a random sequence over a period of a month and a half. Prior to the calorimetric experiment the soils were incubated for 4 days 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 during 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[58]. 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.

Soil bacterial community analysis. The bacterial community structure and diversity were analysed after extraction of soil DNA, amplification and sequencing of the V3-V4 region of the 16S rDNA sequences. 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 triplicates of the semi-urban 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 using a fluorometer (Qubit® *ds*DNA HS) (data not shown).

The V3-V4 variable region of the 16S rDNA sequence 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 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products are checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were pooled in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads, and used to prepare Illumina DNA library. Sequencing was performed by MrDNA-Molecular Research (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq instrument following the manufacturer's guidelines. Sequence data were processed using MR DNA analysis pipeline. In summary, sequences were merged, depleted of barcodes, then sequences < 150bp and sequences with ambiguous base calls were removed. After denoising, operational taxonomic units (OTUs) were generated and chimeras were removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). A total of 1602297 reads were thus obtained, and datasets were normalized at 19047 reads per samples, using R tools.

Analysis of energy content of soluble organic matter. The total energy content of the soluble organic matter was measured by bomb calorimetry[24]. Values for heat of combustion (ΔE) were converted into J $mmol^{-1}$ C (using the mean of the TOC of the respective water extractable organic matter). There was insufficient sample to reliably measure the ΔE for the rural forest soil.

We also measured the composition and diversity of molecular formulae in the soluble organic matter by ultra high resolution mass spectrometry. These data were then used to derive the NOSC of the molecular species contained in the different soluble organic matters[25].

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 Tesla superconducting magnet and an electrospray ionization (ESI) source (Bruker). Freeze-dried water extractable 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[59]. 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 in extemporaneously before the sample analysis using the G24221A Tuning Mix calibration standard from Agilent Technologies (Santa Clara, CA), 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[60], with the version: TRFuFTMSopen07122020. The following formula assigning parameters were employed: the maximum mass error (Δmc = 1010 ppb), $0.3 \leq H/C \leq 2.5$, $0 < O/C \leq 1.25$, $4 \leq C \leq 50$, $0 \leq 13C \leq 1$, $N \leq 5$, $P \leq 1$, $S \leq 3$, singly charged ions in positive mode (max_charge = 1), $-0.5 <$ double bond equivalent (min_DBE), the maximum intensity derivation of C13 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[61].

The NOSC was calculated from the neutral molecular formula estimated from each mass-to-charge ratio detected according to[25].

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

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. The sum of the intensity weighted NOSC of each water extractable organic matter NOSC 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^0_{Cox})[25]. As the ΔG^0_{Cox} of each molecular formula is additive, the bulk ΔG^0_{Cox} of each water extractable organic matter was calculated as follows:

$$\Delta G^0_{Cox} = 60.3 - 28.5 * \text{Sum of the intensity weighted NOSC, at 25°C, 100 kPa. [J per mmol C]} \quad (3)$$

The potential energetic return on investment (ROI) that microbial decomposers can extract during the decomposition of the soluble organic matters was calculated based on Willems et al. [39] and Harvey et al.[24], as follows:

$ROI = \Delta E / \Delta G^0_{Cox}$ (4)

where ΔE (obtained from bomb calorimetry) and ΔG^0_{Cox} (obtained by ESI FT-ICR-MS*)* are both in J $mmol^{-1}$ of C. The ROI represent the ratio between the net energetic benefit and the energy required to activate the oxidation of soil organic C by microbial communities.

Statistics. Rstudio (Version 1.3.1073 - © 2009–2020 Rstudio, Inc) (RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA, http://www.rstudio.com/) 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[62] (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[63] version 1.5.0).

The correlations between the total heat dissipation and the energy return on investment index of each soluble organic matter respectively for each soil have been tested and ploted using the function “ggscatter” (present in the “ggpubr” library version 0.4.0)[64].

Taxonomic diversity indices were calculated with the R script Rhea[65]. The richness was calculated by enumerating the number of variables with a value different from zero. The effective Simpson diversity index was calculated as the inverse of the Simpson diversity index, as follows:

Simpson effective $= 1 / (\Sigma p_i^2)$ (5)

Where p_i is the proportion of the *i*th variable abundance to total abundance in a given sample. The effective Simpson diversity index represents the number of equally abundant variable that would give the same value for the Simpson index[66]. The effective Simpson index has the advantage of been linear compared to the Simpson index[66].

Bacterial composition heatmaps were generated using the plotMRheatmap function in the package “metagenomeSeq”[67] (version 1.30.0).

The relationships between heat dissipation profiles, molecular formulae composition in organic matter and microbial community composition in soil were assessed using Mantel tests on the respective distance matrix with the package vegan[68] (version 2.6-6) and ade4[69] (version 1.7–15).

Declarations

Code availability. The custom R scripts used for data analysis are available from the corresponding author on reasonable request.

Data availability. The datasets generated during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.M.H. and N.N. conceptualized the study. L.J.P.D. collected the data and performed all statistical analyses. C.P. performed the FT-ICR-MS analysis. J.L. performed the bacterial community composition analysis. All authors contributed to manuscript development and revisions.

Additional information

Competing financial interests: The authors declare no competing interests.

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Tables

Table 1a | Results of statistical analyses of the time elapsed for half the total amount of heat to be dissipated.

Test	Parameter	Degree of freedom	Test Statistic	*P*-value
Shapiro-Wilk test of normality			W = 0.75852	4.104E-14
Bartlett's test for homogeneity of variance across groups	soluble OM * Soil	35	K-squared = Inf	2.2E-16
Analysis of Variance of Aligned Rank Transformed Data	Soil	5	F = 186.4989	< 2.22E-16
	soluble OM	5	F = 18.1379	4.9705E-13
	interaction OM : Soil	25	F = 6.3925	3.2681E-12

Table 1b | Results of statistical analyses of the total heat dissipation.

Test	Parameter	Degree of freedom	Test Statistic	*P*-value
Shapiro-Wilk test of normality			W = 0.98616	0.1591
Bartlett's test for homogeneity of variance across groups	soluble OM * Soil	35	K-squared = 57.47	0.00971
Bartlett's test for homogeneity of variance across groups	soluble OM * Soil (log-transformed data)	35	K-squared = 48.523	0.0639
Two-way analysis of variance (ANOVA)	soluble OM (log-transformed data)	5	F = 273.271	< 2E-16
	Soil (log-transformed data)	5	F = 15.734	1.29-11
	interaction OM : Soil (log-transformed data)	25	F = 2.003	0.00774

Table 2 | Mantel tests of heat dissipation profiles with soil bacterial taxonomic composition or with soluble organic matter molecular composition.

	Bray-Curtis dissimilarity indices of heat dissipation profiles [a]	Mantel R	*P*-value [d]
Bray-Curtis dissimilarity indices of soil bacterial taxonomic composition [b]	Rural forest soluble OM	0.9058	**0.005**
	Semi-Urban forest soluble OM	0.9255	0.07
	Urban forest soluble OM	0.9420	**0.025**
	Rural grassland soluble OM	0.9463	**0.049**
	Semi-urban grassland soluble OM	0.9565	0.09
	Urban grassland soluble OM	0.9526	**0.048**
Bray-Curtis dissimilarity indices of soluble organic matter molecular composition [c]	Rural forest soil	0.5174	0.13
	Semi-urban forest soil	0.2879	0.22
	Urban forest soil	0.5515	0.08
	Rural grassland soil	0.4398	0.12
	Semi-urban grassland soil	0.6014	0.08
	Urban grassland soil	0.5959	0.09

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

[b] Bray-Curtis dissimilarity indices calculated with normalised 16S rRNA gene data between each soil.

[c] Bray-Curtis dissimilarity indices calculated with normalised FT-ICR-MS data between each soluble organic matter.

[d] Significant differences ($P < 0.05$) are indicated in bold.

Table 3 | Diversity and energy return on metabolic investment indices of soluble organic matter.

Soluble OM	Molecular formulae [a]		NOSC [b]		DE [e] J (mmol C)$^{-1}$	Sum of intensity weighted NOSC [f]	DG^0_{Cox} [g] J (mmol C)$^{-1}$	ROI [h] (DE / DG^0_{Cox})
	Richness [c]	Simpson effective [d]	Richness [c]	Simpson effective [d]				
Rural Forest	2007	139	641	52	NA	- 0.30	68.75	NA
Semi-Urban Forest	2147	82	699	34	542.41	- 0.44	72.88	7.44
Urban Forest	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
Semi-Urban 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

[a] The molecular formulae have been estimated by the algorythm TRFu[60] from the masses-to-charge detected by ultra high resolution mass spectrometry for each soluble organic matter (n = 1).

[b] The nominal oxidation state of C (NOSC) have been calculated from each neutral molecular formulae using the equation presented by LaRowe & Van Cappellen[25] for different soluble OM analysed by ultra-high resolution mass spectrometry (n = 1).

[c] The Richness is calculated by respectively enumerating the molecular formulae or the NOSC present in each soluble organic matter.

[d] The effective Simpson diversity index of molecular formulae and NOSC profiles. This represents the number of equally abundant molecular formulae or NOSC that would give the same value for the Simpson index[66]. The effective Simpson index has the advantage of been linear compared to the Simpson index[66].

[e] Values of the heat of combustion in Joules per mmol C liberated during the complete oxidation of the freeze-dried soluble organic matter measured with bomb calorimetry (n=1). The heat of combustion (DE) represents the energy content in the organic matter. The value for the soluble organic matter of rural forest couldn't be determined due to a lack of freeze-dried material.

[f] Sum of intensity weighted values of the nominal oxidation state of carbon (n = 1) that is calculated using the TRFu algorithms developped by Fu et al.[60].

[g] Values of Gibbs free energy of oxidation half reactions at standard conditions ($DG°_{Cox}$) estimating for each souluble organic matter using the sum of intensity weighted NOSC and the equation presented by LaRowe & Van Cappellen[25] (n=1).

[h] Energy return on investment (ROI) which soil microbial communities potentially make during the transport and catalysis of each soluble organic matter. The ROI represents the benefit to cost ratio of transforming organic matter. We proposed to calculate the ROI by dividing the energetic content of each soluble organic matter (DE) by their Gibbs free energy of oxidation half-reaction ($DG°_{Cox}$).

Figures

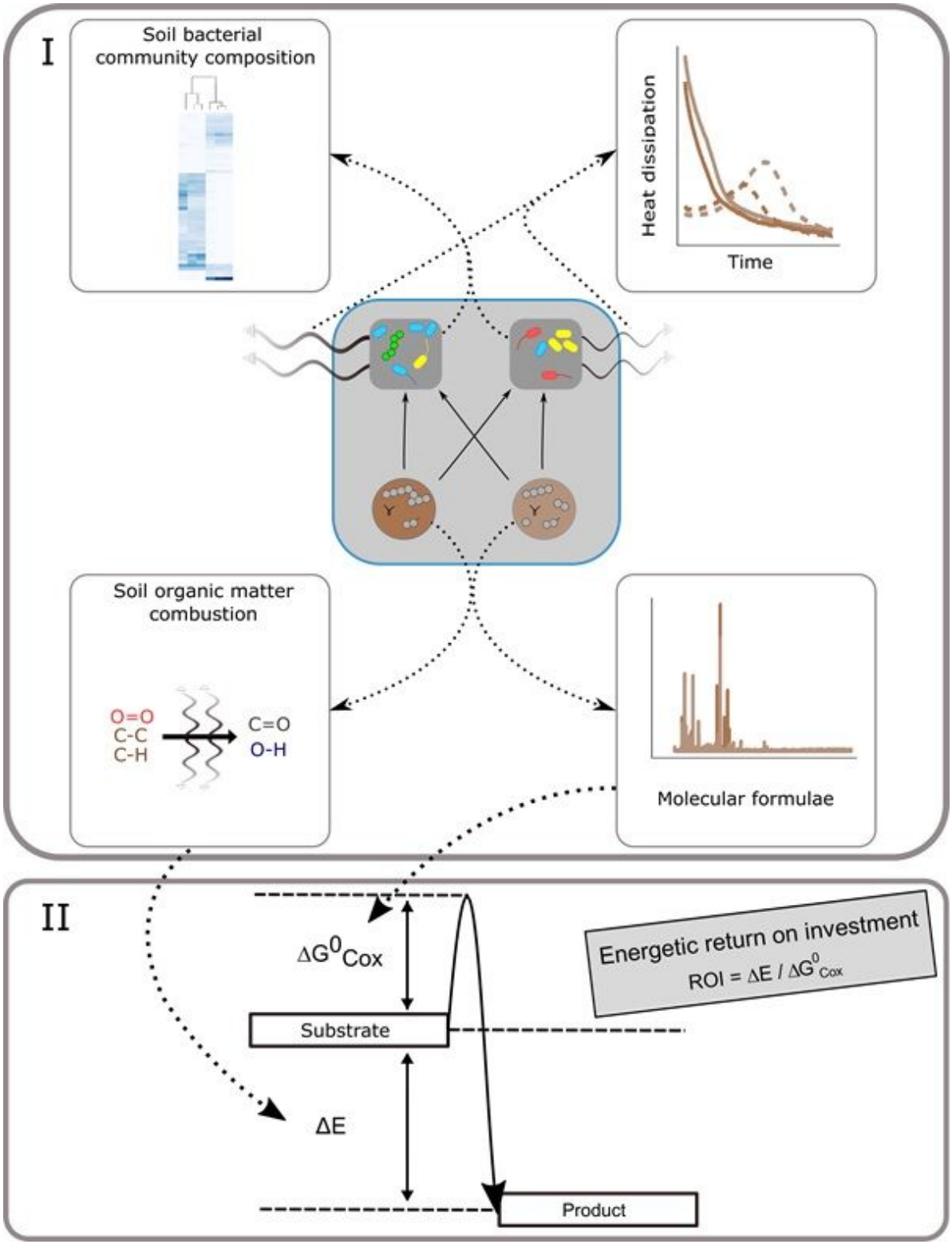

Figure 1

Conceptual representation of the experimental design. (I) A range of soluble soil organic matter containing thousands of different molecules but the same quantity of organic C were added cross-wise to soils. Soils harboured distinct bacterial communities. The metabolic responses of microbial communities to the addition of soluble organic matter were determined as heat dissipation dynamics during a 24 h period. (II) The energetic return on investment (ROI) was calculated as the ratio between the total net

energy available (DE) and the weighted average activation energy (DG0Cox). The total net energy available (DE) was measured by bomb calorimetry24. The energetic barrier (DG0Cox) of organic molecules is assumed to increase when its average nominal oxidation state of C (NOSC) decreases25. In addition, we assume that DG0Cox 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 is taken into account)70.

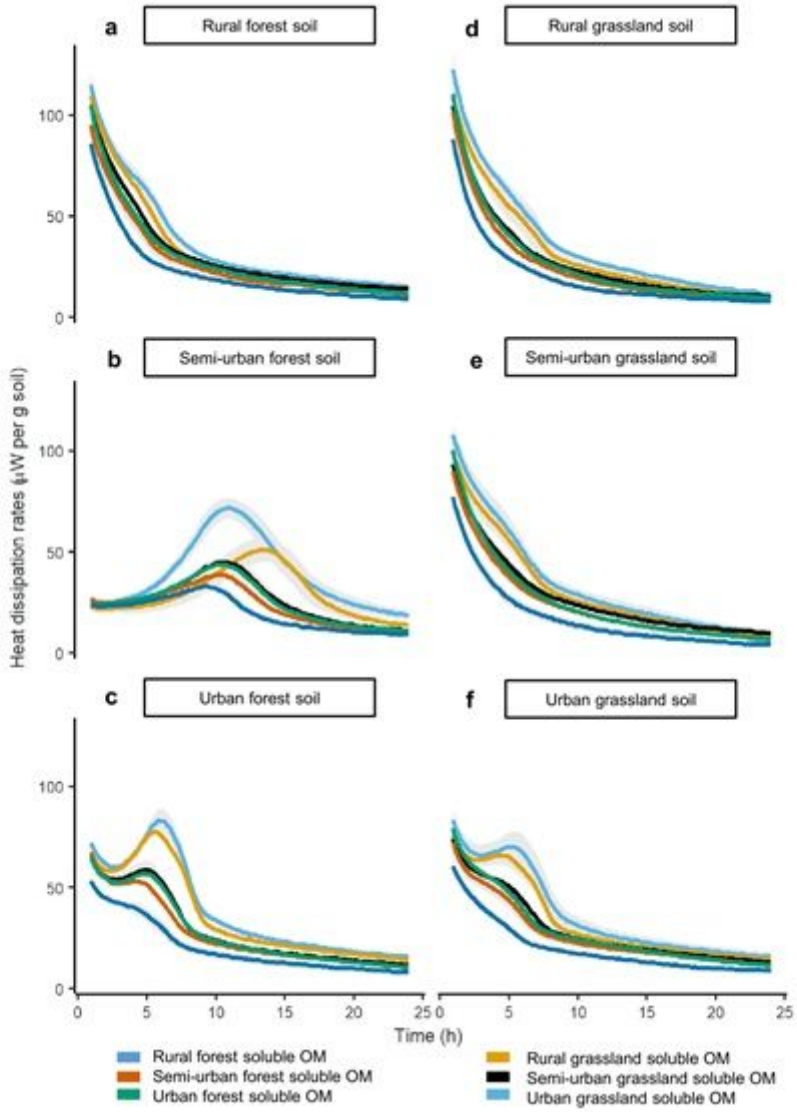

Figure 2

Heat dissipation patterns in (a) rural forest, (b) semi-urban forest, (c) urban forest, (d) rural grassland, (e) semi-urban grassland or (f) urban grassland soils after the addition of 0.3 mg organic carbon from each of the 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.

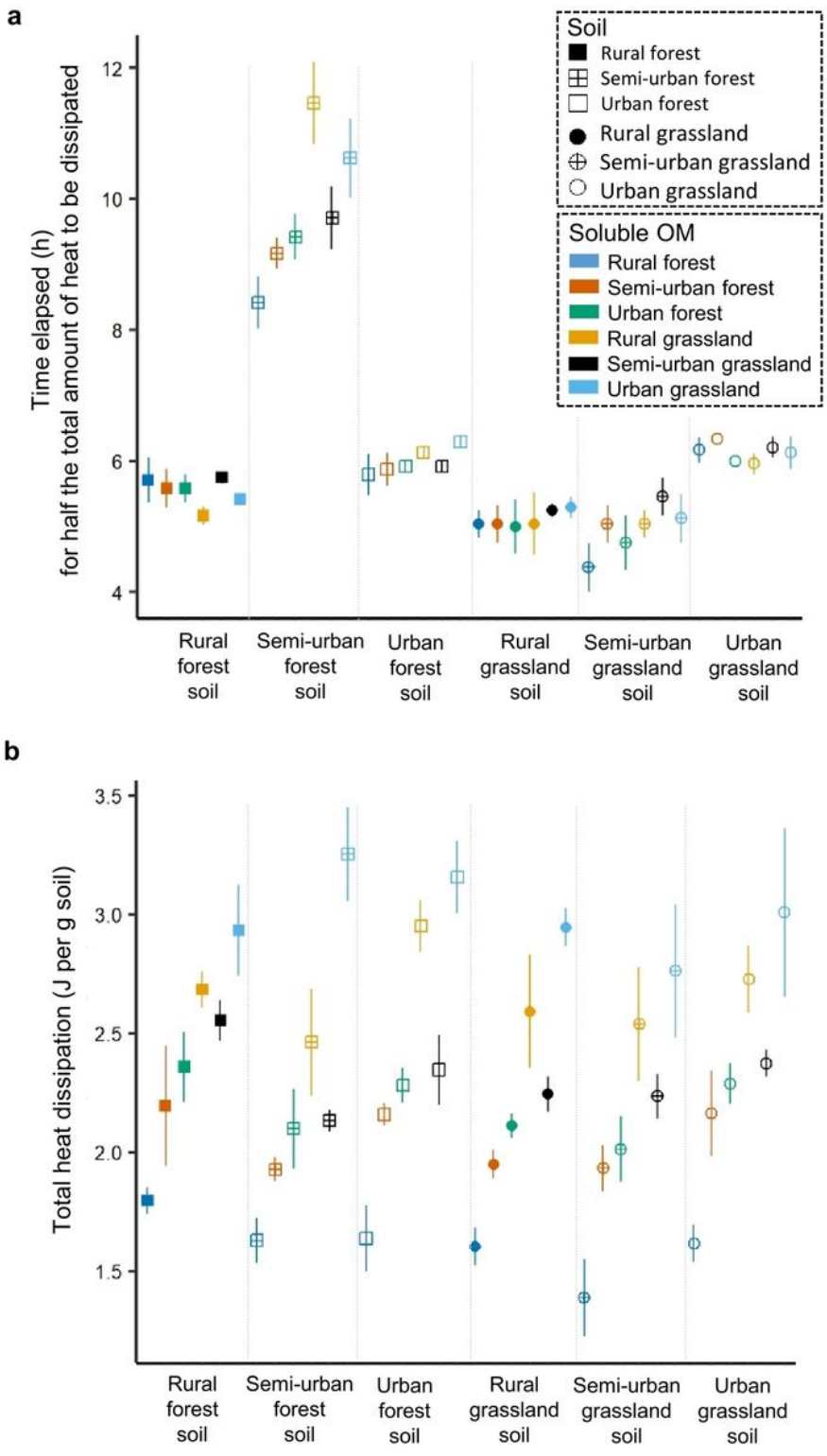

Figure 3

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

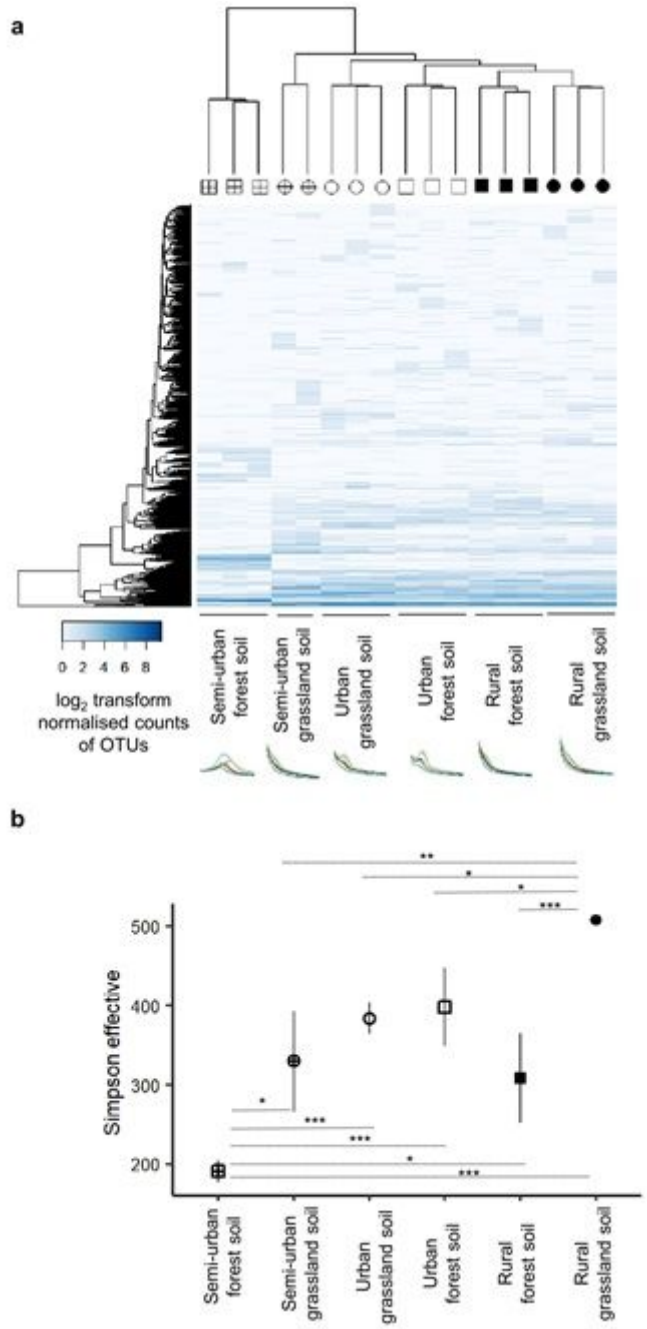

Figure 4

Differences in soil bacterial taxonomic composition and effective Simpson diversity index. (a) Heat maps of the bacterial OTU abundances (log2 transform normalised counts) and hierarchical clustering of the bacterial communities in soils. The curves represent the different heat dissipation profiles in each soil. (b) Effective Simpson diversity index of the bacterial communities. Each symbol represents the mean ± one standard deviation for each soil groups (n = 3 except for the semi-urban grassland soils where n = 2). Tukey's HSD significance: *, P < 0.05, **, P < 0.01; ***, P < 0.001.

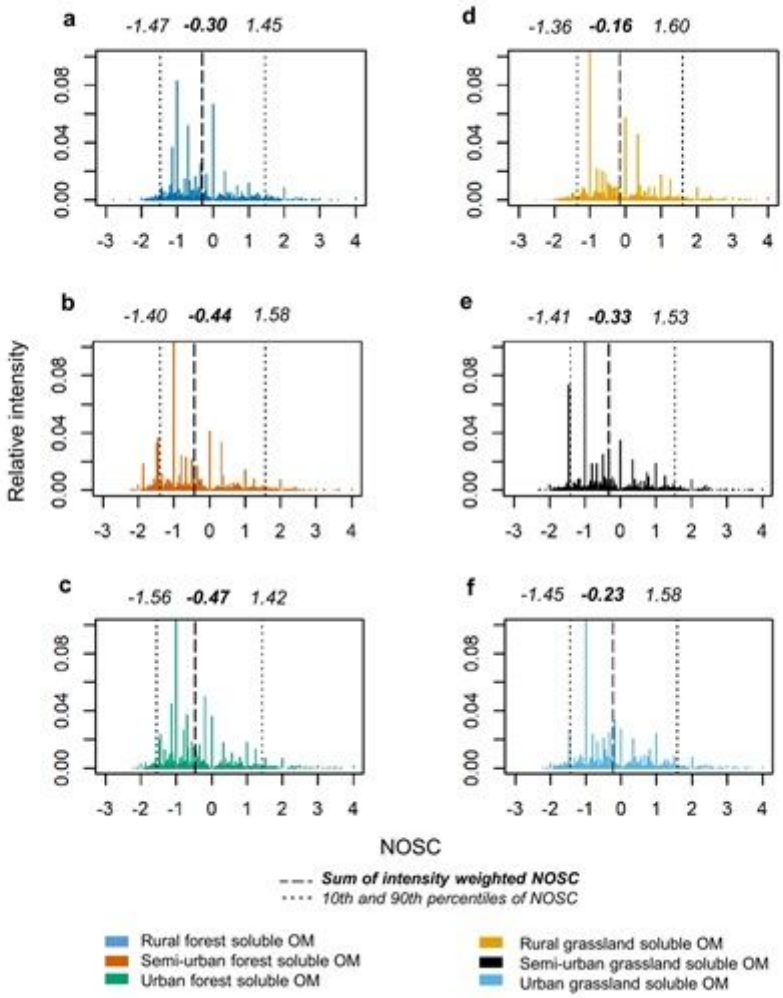

Figure 5

Distribution of the relative intensities of nominal oxidation state of carbon (NOSC) in soluble organic matter (OM) from (a) rural forest, (b) semi-urban forest, (c) urban forest, (d) rural grassland, (e) semi-urban grassland, (f) urban grassland. A Kolmogorov-Smirnov test on the NOSC data of each soluble organic matter indicated that the distribution of NOSC from the urban forest soluble organic matter was significantly different from the one from the rural meadow soluble organic matter (D = 0.090, P = 0.015).

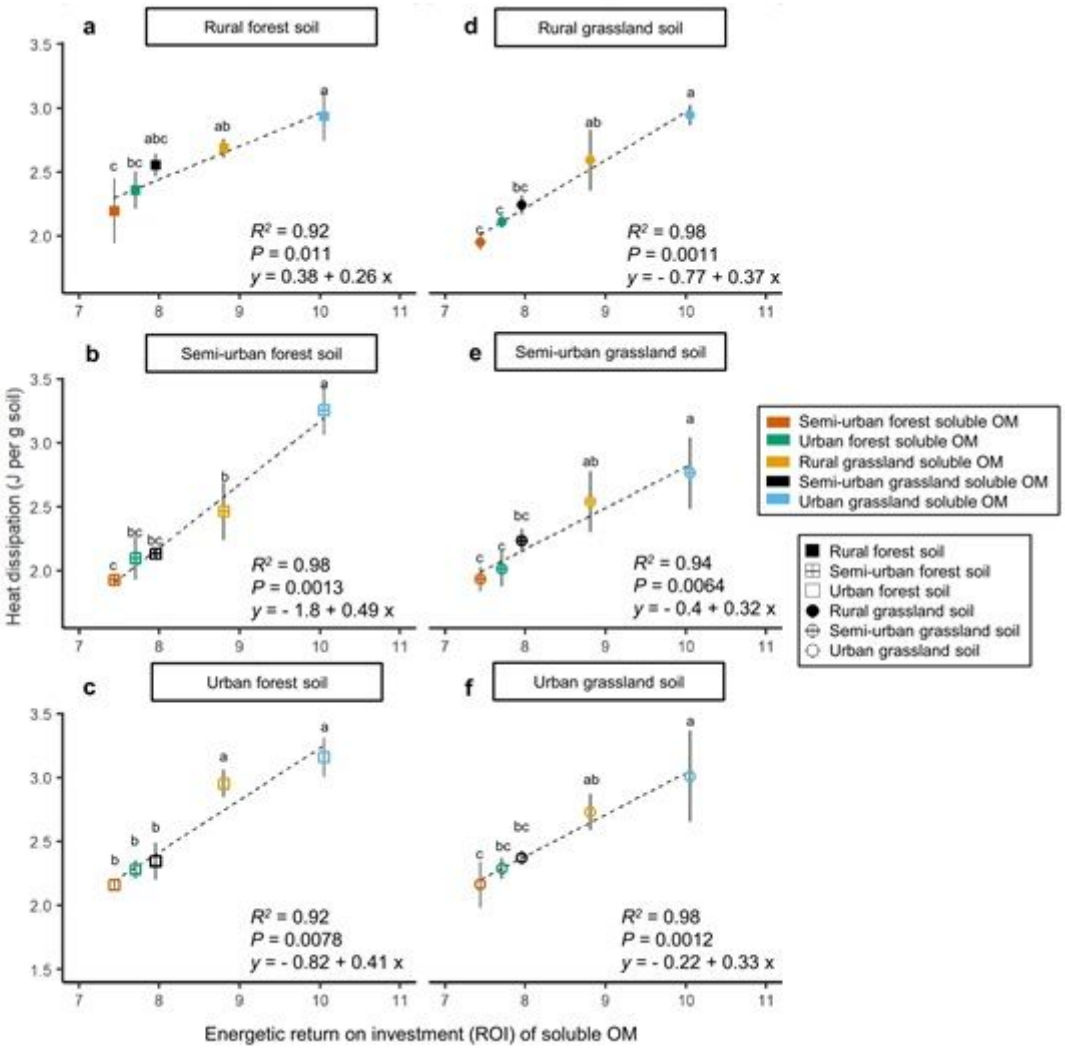

Figure 6

Relationships between the total heat dissipation and the potential energetic return on investment: (a) rural forest, (b) semi-urban forest, (c) urban forest, (d) rural grassland, (e) semi-urban grassland or (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).

Supplementary Files

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