Université de Montréal

Evaluating the environmental impact of vertebrate body decomposition:

A soil biogeochemical perspective

Par

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Programme de sciences biomédicales, Faculté de médecine

en extension à l'Université du Québec à Trois-Rivières

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Résumé

La matière organique du sol (MOS) est un important réservoir d'énergie et de nutriments dans les environnements terrestres. L'équilibre entre les apports, la transformation et le recyclage de la MOS est essentiel pour maintenir la santé des écosystèmes. Le parcours de la MOS dans l'écosystème est contrôlé par des interactions réciproques entre sa chimie et les communautés bactériennes. Ces dynamiques sont bien documentées pour la MOS issu de la matière végétale, mais celles provenant des vertébrés restent peu étudiées. Outre un apport concentré de la MOS, la décomposition des vertébrés libère aussi des bactéries entériques et des substances inorganiques (ex. sels, minéraux), produisant une zone de sol biochimiquement perturbée appelée l'îlot de décomposition cadavérique (IDC). L'augmentation de la mortalité animale due au changement climatique et l'essor de la recherche médico-légale sur la décomposition ont suscité des inquiétudes vers l'impact environnemental de la décomposition des vertébrés. Cette thèse explore ces enjeux en étudiant l'interaction entre la chimie de la MOS et les bactéries au sein des IDCs, en évaluant : (1) la quantité et les caractéristiques chimiques de la MOS et de ses fractions solubles, (2) l'activité, le métabolisme et la structure des communautés bactériennes, (3) les liens entre la chimie de la MOS et les réponses bactériennes, et (4) la manière dont ces dynamiques diffèrent selon les conditions saisonnières et régionales. Trois études ont été menées sur des IDCs (porcs ou humains) dans une forêt tempérée (Québec, Canada) puis dans une savane tropicale (Hawaï, États-Unis).

Au cours de ces études, les effets des IDCs se limitaient aux sols peu profonds (<14 cm) dans un rayon d'échantillonnage de 20 cm autour des restes. Les analyses chimiques ont révélé des apports accrus de composés labiles issus surtout des tissus et sous-produits microbiens. Ceux-ci ont été associés à une augmentation de la respiration bactérienne et du métabolisme des glucides et acides carboxyliques. La croissance bactérienne a toutefois été inhibée, suggérant une allocation de la MOS vers la respiration plutôt que la biomasse. De plus, les apports labiles étaient corrélés à l'émergence de bacilles, de clostridies et de gammaprotéobactéries, néanmoins chacun de ces taxons contribuant différemment aux changements d'activité et de fonction de la communauté. Les pics de matière labile et de réponses bactériennes ont diminué avec le temps,

mais le traitement microbien de la MOS a entraîné la formation de composés humiques persistants (>1 an). Les apports et les transformations microbiennes de la MOS se sont étonnamment poursuivis dans des conditions froides, mais à un rythme et ampleur réduite. De plus, la décomposition dans différentes régions géographiques a mené à des changements similaires dans la chimie de la MOS, néanmoins les bactéries des sols tropicaux semblaient plus sensibles aux apports labiles. Ces résultats mettent en évidence le rôle des apports de MOS dans les IDC, agissant à la fois comme une ressource et une perturbation écologique. Cela permettra d'élaborer des stratégies de gestion environnementale ainsi qu'a contribué à une meilleure intégration des apports vertébrés dans des cycles biogéochimiques.

Mots-clés : Îlot de décomposition cadavérique, Apports vertébrés, Matière organique du sol, Cycles biogeochimiques, Taphonomie forensique, Métabolisme bactérien, Communautés bactériennes, Composés labiles, Composés humiques, Variabilité biogéoclimatique.

Abstract

Soil organic matter (SOM) is an important reservoir of energy and nutrients in terrestrial environments. The balance between SOM inputs, transformation and cycling is crucial for maintaining ecosystem health. Each step of SOM's journey within an ecosystem is controlled by reciprocal interactions between its chemistry and bacterial communities. While these dynamics are well characterized in SOM derived from leaf litter, contributions from decomposing vertebrates remain understudied. In addition to being a concentrated source of SOM, decomposing vertebrates also release enteric bacteria and inorganic substances (e.g. salts, minerals). These altogether lead to a biochemically disrupted area of soil known as the Cadaver Decomposition Island (CDI). Rising animal mortalities from climate change and recent increases in forensic decomposition research have led to concerns over the environmental impact of vertebrate decomposition. The following dissertation addresses these concerns by investigating the interplay between SOM and bacteria within CDIs. This was achieved by exploring spatiotemporal patterns in: (1) the quantity and chemical composition of SOM and its soluble fractions, (2) bacterial activity, metabolism, and community structure, (3) the links between SOM chemistry and bacterial responses, and (4) how these dynamics differ across seasonal and regional conditions. Three studies were conducted on vertebrate CDIs (pig or human) within a temperate forest (Québec, Canada) or tropical savannah (Hawaii, USA) ecosystem.

Across all studies, CDI effects were confined to shallow soils (<14 cm depth) within a 20 cm sampling radius from the remains. SOM analyses revealed heightened inputs of labile compounds that were largely derived from tissues and microbial by-products. These compounds drove increased rates of bacterial respiration and carbohydrate/carboxylic acid metabolism. Bacterial growth was however suppressed, thus suggesting a greater shuttling of SOM towards respiration rather than bacterial biomass. Labile inputs were also correlated with the emergence of Bacilli, Clostridia, and Gammaproteobacteria. Each of these taxa were found to differentially contribute the community's activity levels and function. Peaks in labile SOM and bacterial responses diminished over time, yet the microbial processing of SOM resulted in the formation of persistent (>1 year) humic-like compounds. Remarkably, SOM inputs and bacterial

transformations continued through cold winter conditions, but at reduced rate and magnitude. Decomposition across geographic regions produced equivalent changes in SOM chemistry, however tropical soil bacteria appeared more metabolically reactive to the inputs. These findings help create a mechanistic understanding of how SOM inputs in CDIs act as both an ecological resource and disturbance across seasonal and regional conditions. This will inform environmental management strategies for areas that deal with decomposing animal or human remains, as well as help to better integrate vertebrate inputs into biogeochemical cycles.

Keywords : Cadaver decomposition island, Soil organic matter, Vertebrate inputs, Biogeochemical cycling, Forensic taphonomy, Bacterial metabolism, Bacterial communities, Labile compounds, Humic compounds, Biogeoclimatic variability.

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List of abbreviations

ADD	Accumulated degree days
ASV	Amplicon sequence variant
AWCD	Average well colour development
BA	Bacterial abundance
BGE	Bacterial growth efficiency
BIX	Biological index
BP	Biomass production
BR	Bacterial respiration
CDI	Cadaver decomposition island
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EEM	Excitation-emission spectra
Em	Emission wavelength (nm)
Em_{max}	Maximum emission wavelength
Ex	Excitation wavelength (nm)
Ex_{max}	Maximum excitation wavelength
FI	Fluorescence index
HIX	Humification index
HS	Humic substances
HTF	Human taphonomic facility
LME	Linear mixed effects
MAOM	Mineral-associated organic matter
NMDS	Non-metric dimensional scaling
PARAFAC	Parallel factor analysis
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
PLS-R	Partial least squares regression

PMI	Postmortem interval
POM	Particulate organic matter
REST[ES]	Research in Experimental and Social Thanatology / *Recherche en sciences thanatologiques, expérimentales et sociales*
RU	Raman units
SD	Standard error
SOM	Soil organic matter
S_R	Spectral slope ratio
UQTR	Université du Québec à Trois-Rivières
VIP	Variable of importance to projection

List of symbols

$\delta^{13}C$	Ratio of ^{13}C to ^{12}C
$\delta^{15}N$	Ratio of ^{15}N to ^{14}N
n	Sample size
p	Probability value
R^2	Coefficient of determination
Q^2	Predictive squared correlation coefficient
Df	Degrees of freedom
F	F statistic value
RV	Vector correlation coefficient

“The dead bird in the back yard under the spruce tree

I have passed many days now while slowly

all the ugly innocent necessary work of nature

is carried on by beetles, ants, blowflies –

all those immediate molecular transpositions

that ensure

the endless procession of pine needles, new eggs,

new birds and in their turn

new deaths”

– Loren Eiseley, Notes of an alchemist (1972)

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The completion of this dissertation is personal milestone and a testament to my dedication and efforts. I am however profoundly aware that this accomplishment is not a solitary one. This dissertation, in addition to the experiences, knowledge and skills accrued throughout my doctorate, would have not been possible without the following individuals.

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I wouldn't be where I am today without my loving and incredibly supportive family. You allowed me to pursue this route and never questioned my morbid curiosities, rather you encouraged them. You made me comfortable in my weirdness and showed me how it is strength

rather than a flaw. You were there when I was struggling, mentally and financially, and you did what was in your power to help smooth things over. I am extremely privileged to have had your help, comfort and encouragement. I owe a big part of this dissertation to you.

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Preface

Back in 2018-2019, I was following the establishment of Canada's first human taphonomic research facility. This was done within the scope of my MSc project, where I aimed to identify key processes and considerations that contributed to the successful opening of the facility. The sharing of my findings was intended to help future researchers and institutions navigate the complexities associated with establishing their own sites. This was in effort to help improve the success rate of new facilities and the global distribution of forensic decomposition research. Throughout this process, numerous inquiries were posed by citizens and several levels of government (municipal, provincial) on the potential environmental risks associated with the presence of decomposing human and/or animal remains. The literature unfortunately contained very little information on the matter, particularly with regards to the unique conditions of a temperate Canadian environment. It became apparent that this lack of knowledge could be a significant barrier for future facilities. I decided to address this gap myself by transferring directly into PhD.

Soon after starting, it became apparent that the environmental impact of vertebrate decomposition was a concern beyond just forensic taphonomy. This was personally expressed to me by individuals working in the green burial and the livestock industries. It is here that I decided to integrate a more ecological narrative and expand the dissemination of my findings to both forensic and non-forensic outlets. This effort not only allowed me to respond to the needs of multiple sectors but also broadened the perspective through which I analyzed and interpreted my results.

The interdisciplinary nature of this project is reflected in the structure of the following dissertation, which consists of an introduction and literature review (Chapter I), three standalone scientific articles (Chapters II–IV), and a conclusion (Chapter V). Each article is formatted according to the requirements of the intended journal. Chapter II has been published in the *Journal of Geophysical Research: Biogeosciences*, Chapter III has been submitted to *Forensic Science International*, and Chapter IV is in preparation for submission to the *Journal of Environmental Microbiology*. My director, Prof. François Guillemette (UQTR), and co-director,

Prof. Shari Forbes (University of Windsor), are listed as co-authors on each article. They secured the necessary funding and resources for this project, provided financial support, and offered invaluable guidance in project conceptualization, method development, and data validation. Chapter IV also includes Dr. David Carter, who welcomed me to study and sample the soils around decomposing pig carcasses at Chaminade University of Honolulu (Hawaii). This opportunity enabled me to address one of the main points of my dissertation; being the role that biogeography plays in shaping the impact vertebrate decomposition has on soil systems. This chapter also included co-author Prof. Hugo Germain (UQTR), who generously off-set some DNA sequencing costs by doing the PCR amplification (performed by Mélodie Plourde, UQTR) through his lab. Prof. Germain's also assisted with validating the bioinformatics (performed by Théo Devèze, UQTR) and community data analyses. All co-authors moreover contributed valuable feedback on manuscript drafts. All other work required for the completion of this dissertation was completed by me, Emily Pecsi.

Chapter 1 – Introduction

Premise and importance

Upon their death, vertebrates act as a unique and valuable resource to terrestrial systems. Animals sequester energy and nutrients within their tissues, which upon their decomposition, are released into the ground as a concentrated pulse of soil organic matter (SOM) (Barton & Bump, 2019). The resources provided by decomposing animal biomass (carrion) can positively influence the surrounding environment as it helps support food webs and biodiversity (Barton et al., 2013, 2019). At the same time, decomposing vertebrates can be immensely disruptive. The concentrated influx of carrion materials have been observed to trigger ephemeral to long-lasting changes in soil biochemistry (Carter et al., 2007; Cobaugh et al., 2015). These localized changes, paired with the enhanced resource availability, collectively contributes to landscape heterogeneity in what is known as the Cadaver Decomposition Island (CDI). However, the mechanisms driving the turnover of carrion inputs in CDIs and their downstream effect on ecosystem processes (e.g. primary production) have been minimally investigated (Barton et al., 2019; Fiedler et al., 2023). As a result, the degree in which carrion act as a resource or disturbance is poorly defined.

The environmental implications of decomposing vertebrates and their CDIs is an emergerging interest in both the fields of ecology and forensic taphonomy. For the most part, ecologists have previously focused on carrion's role in supporting food-webs and macrofauna biodiversity (Barton et al., 2013; Brundage, 2021). Due to climate change and anthropogenic activities, there have been increases in the frequency and magnitude of mass mortality events in both wildlife and livestock populations (Bartel et al., 2024). Estimate from Fey et al. (2015) suspect that there has been one additional event per year since the 1940s, indicating an exponential increase in mortalities. Declines in scavenger and negrophagous species have also meant that more carrion materials are directly entering the surrounding soils (Newsome et al., 2021). Ecologists are now raising concerns on the potential influence carrion inputs may have on local energy/nutrient budgets and ecologial functioning (Barton et al., 2019). Similar inquiries have been made in forensic taphonomy; a field dedicated to studying the progression and outcome of

human decomposition for applications in suspicious death investigations (e.g. victim decovery, postmortem interval estimation). Controlled studies on donated human remains are securely and ethically performed in outdoor sites known as human taphonomic facilities (HTF) (Pecsi et al., 2020). The first HTF was established in the 1980s at the University of Tennesee, Knoxville. Since then, there have been thirteen such sites that have opened globally (USA-10 , AUS-1, CAD-1, NED-1), with the most recent one lauching at Louisiana State University (Louisiana State University, 2025; Pecsi et al., 2020; Vidoli et al., 2017). HTFs can vary in size, ranging from small plots (e.g. 1600 m^2 for RESTES, UQTR) to expansive terrains (e.g. 26 acres for FARF, Texas State) (Gocha et al., 2022; Pecsi et al., 2020) . The worldwide increase in the number, scale, and operational activity of HTFs has resulted in an all-time high in the number of decomposing bodies present within these sites. Some are beginning to question whether the deposition of donor bodies affects the environmental integrity of HTFs, as this may lead to HTF-specific decomposition processes that not representative of real-life scenarios (Carter & Tibbett, 2008b; Damann et al., 2012). Ecologists and forensic taphonomists alike are moreover interested in verterate decomposition and its environmental impact under cold seasonal conditions, which have historically been understudied (Iancu et al., 2024; Situnayake et al., 2025). To address the needs of both fields, we must gain an understanding of how soils under varying climates respond to decomposition products and the extent in which their effects can be naturally mitigated.

Microbes are essential recyclers. Their activity and function shape the breakdown, transformation and distribution of SOM inputs (Hermans et al., 2017). Bacteria particularly play a key role in these processes, as their metabolic diversity and low trophic position allows them to shuttle an assortment of compounds through the food chain and biogeochemical cycles (Potapov et al., 2021; Prosser, 2006). But not all SOM is treated equally. Based on leaf litter studies, the quantity and molecular complexity of SOM compounds will dictate the ease and rate of their degradation (i.e. lability). In return, compounds with varying levels of lability will yield different amounts of energy that can be used to further fuel microbial processes and community structure. Microbes can furthermore enhance the molecular complexity and stability of SOM, thereby decreasing its lability, through enzymatic reactions and the release of bulky biomass components (Balser, 2004; Chapin et al., 2002; Guggenberger, 2005). This bi-directional relationship between

SOM and bacteria is crucial for balancing the flux of resource inputs. Dysregulations can lead to the excessive accumulation or loss of SOM, which can ultimately result in environmental decline (Chapin et al., 2002). SOM-bacterial dynamics following the decay of vegetation have been well established. Although informative, plant-based findings cannot be directly applied to vertebrates due to drastic differences in mass, decomposition rates, stoichiometry (e.g. C:N) and ecology (i.e. scavenging/necrophagy vs herbivory) (Barton et al., 2013, 2024; Carter et al., 2007; DeBruyn et al., 2024). Gaining a similar understanding in CDIs can however help ecologists and forensic taphonomists evaluate how carrion-associated changes in SOM chemistry influence bacterial responses and their cycling abilities. This will provide a mechanistic understanding of the drivers controlling the dispersion, persistence and impact of carrion-derived SOM, which will ultimately inform the development of environmental management strategies for both HTFs and animal mortality events.

Research questions

The lack of CDI-specific information on SOM-bacterial dynamics limits our current understanding of the environmental impacts of vertebrate decomposition. This dissertation begins to address these shortcomings by answering four key questions:

1) Do vertebrate decomposition inputs have an effect on the SOM pool and its cycling?
2) Does decomposition-related changes in SOM influence bacterial communities and their ecological function?
3) What is the spatial and temporal extent of decomposition-related disruptions in SOM and soil bacteria?
4) Do decomposition-related disturptions in SOM-bacterial dynamics differ over seasonal conditions and geographic regions?

Dissertation organization

The following doctoral dissertation aims to evaluate the environmental impact of vertebrate decomposition in terrestrial ecosystems through the assessment of SOM-bacterial dynamics. The project integrates biogeochemistry, microbial ecology and forensic taphonmy to

explore the natural processes that underpin the cycling of organic vertebrate decomposition products. The section below consists of a literature review which summarizes in greater detail the current concepts, techniques and knowledge gaps that are associated with this project's subject matter. This forms the conceptual framework in which this dissertation's objectives are based on.

The proceeding chapters (II-IV) present the experimental studies that were conducted in order to fulfill the project's overarching aim and research questions. These chapters are formated as standalone scientific articles that are intended for publication. Chapter II was performed as a pilot study on pig CDIs in a temperate mixed forest of Trois-Rivières, Québec. It focused on making preliminary observations and correlations between SOM chemistry and bacterial responses. It also intendended to examine SOM and bacterial patterns over the progression of temperate seasons (summer > autumn > spring). Chapter III consists of a human study performed at the REST[ES] facility in Bécancour, Québec. This produced a set of human-specific observations that are more relevant to HTFs and forensic applications. Additional techniques were integrated into this study in order to further trace SOM inputs and its cycling pathways. Chemical and bacterial patterns were also assessed acording to deposition season, whether the donors were placed under warm (summer, spring) or cold (autmn, winter) seasonal conditions. Chapter IV explores how bacterial community structure branches the changes in SOM chemistry and bacterial responses. This study involved pig CDIs formed within a Hawaiian tropical savanna ecosystem, which further facilitated the assment of regional differences in SOM chemsitry and bacterial responses through comparisons with Chapter II. Lastly, Chapter V presents genereal conclusions from the ensemble of studies before discussing potential limitations and future research avenues.

Literature review

Principles of SOM chemistry and cycling

Soil organic matter (SOM) is a complex mixture of biomolecules and polymer-like substances that originate from the decomposition and cycling of plant, animal and microbial residues (Horwath, 2015). It serves as an important pool of energy (i.e. carbon) and nutrients for

terrestrial microorganisms, whose activity ultimately contributes to the maintenance of biogeochemical cycles and ecological processes (e.g. primary productivity) (Buscot, 2005; Guggenberger, 2005). SOM moreover plays an important role in soil structure, pH buffering and the retention of numerous substances (i.e. water, xenobiotics) (Weil & Brady, 2017). The breakdown and reintegration of SOM into trophic pathways and biogeochemical cycles is dependent on a variety of physicochemical factors, most notably resource quality and availability, as well as microbial activity, function and diversity.

Due to the complexity of bulk SOM, it must first be divided into smaller fractions in order to be more easily studied and characterized. SOM can be subdivided based on its functional properties, resulting in three major fractions: particulate organic matter (POM), dissolved organic matter (DOM) and mineral-associated organic matter (MAOM). The first fraction of POM is a mixture of large (> 0.45 μm) and minimally degraded compounds or biopolymers that are sourced directly from biological residues (Lehmann & Kleber, 2015; Smreczak & Ukalska-Jaruga, 2021). Their large size generally limits microbial assimilation, thus additional break-down is first needed through the action of exoenzymes (et al., 2002a). Once sufficiently degraded, compounds can then enter the DOM fraction. The enhanced solubility of DOM permits its easier diffusion across both the soil profile and microbial membranes, thereby enhancing its assimilation and biological reactivity (Marschner & Kalbitz, 2003). As a result, the DOM fraction is most implicated in the microbial processing and turn-over of SOM (Chenu et al., 2014). DOM can be further sub-divided according to its elemental composition, the largest group (~67%) being dissolved organic carbon (DOC) (Bolan et al., 2011). The DOC fraction can be easily quantified in soil extracts through the evolution of CO_2 from chemical oxidation (e.g. persulfate) or combustion (McKenna & Doering, 1995). It is therefore commonly employed as a proxy for overall DOM and SOM quantities (James & Harrison, 2023). At any point in its existence, POM and DOM can chemically adsorb to mineral surfaces on soil particles to form microbially inaccessible MAOM (Lehmann & Kleber, 2015; Luo & Xu, 2023), which is further described below under “Physicochemical stabilization”. This adsorption is not permanent and can revert following changes in soil conditions. This creates a continuous exchange in the degradability and fractionation of SOM known as the Soil Continuum Model (Lehmann & Kleber, 2015).

Quantity is a useful measure for capturing fluxes, but it fails to identify the biological utility and transformation of the SOM pool. Fortunately, this can be evaluated based on the molecular complexity, or quality, of DOM compounds. For instance, lignin and tannins from plant litter are considered low-quality, as they are more resistant to degradation due to their irregular chemical structure, high degree of conjugation and aromaticity (Fellman et al., 2010; Marín-Spiotta et al., 2014). Complex, stable, aromatic molecules are difficult to breakdown since the majority of microbially produced enzymes are unable to target the irregularly shaped active sites and/or degrade the double-bonds contained within aromatic rings (Chapin et al., 2002). In contrast, simple and low-molecular weight amino acids and carbohydrate monomers are considered high-quality and labile, thanks to their energy-rich content and easily biodegradable structure (Marschner & Kalbitz, 2003). Such labile compounds primarily originate from microbial metabolic byproducts, a protein-rich allochthonous source (e.g. animal residues) and/or the degradation of higher-order molecules (Fellman et al., 2010; Gmach et al., 2020; Hood et al., 2007).

Traditional views of soil science assumed that some low-quality species of SOM existed in the form of humic substances (HS) as a result of humification; a collection of processes that lead to the formation and accumulation of highly modified and chemically stable compounds (e.g. humic acids, humin) that are resistant to further decomposition. The characteristic high molecular weight, structural complexity, hydrophobicity and aromaticity of HS were thought to confer turn-over times of hundreds to thousands of years (Guggenberger, 2005). The formation of HS was mainly ascribed to the spontaneous polymerization of smaller subunits (Balser, 2004; Chapin et al., 2002). For example, quinone groups in oxidized plant phenols would condense with labile microbial peptides to create larger molecular structures in what is known as the “Phenol-Protein Theory” of humification (Horwath, 2015; Zavarzina et al., 2021). HS were in parallel assumed to accumulate in soils through the action of selective preservation, which occurs when microbes preferentially target labile SOM and leave behind less desirable humic compounds. In light of emerging evidence, SOM stability is increasingly understood as a product of microbial accessibility rather than a feature of molecular complexity. Modern approaches assume that all SOM is subject to microbial degradation at one point or another, regardless of chemical recalcitrance. However, it becomes stabilized once it is physically inaccessible to microbial attack upon mineral adsorption

(i.e. MAOM) or occlusion within soil aggregates. A growing number of molecular-level analyses even suggests that microbial biomass components (i.e. necromass) and enzymes are more susceptible to forming organo-mineral associations due to their lower molecular weight, relative to vegetally-sourced compounds, polar functional groups (e.g. amino, carboxyl, hydroxyl) and sometimes amphiphilic properties (e.g. proteins) (Cotrufo et al., 2012; I. Kögel-Knabner, 2016; Kleber et al., 2007). The true source of SOM stability likely lies in-between these modern and traditional views, where the molecular recalcitrance and physical accessibility of SOM are in constant flux within and between fractions as the result of both chemical and biological mechanisms. Either way, both schools of thought recognize microbes as an important player in SOM stabilization and recalcitrance.

Information on DOM quality can be inferred using cost-effective spectroscopic techniques. This is possible for two reasons. First, a sub-fraction of DOM contains optically active moieties that produce distinct absorbance and fluorescence spectra given a compound's molecular structure. From this, researchers have been able to develop a series of optical indices that broadly describe DOM molecular characteristics, lability and source (**Table 1.1**). Second, the development of Parallel factor analysis (PARAFAC) has facilitated the mathematical deconvolution of complex 3D fluorescent excitation-emission spectra (EMMs) into independent and easily interpretable signals (i.e. components). This approach has enabled key fluorescent peaks to be identified and associated with known compounds (**Table 1.2**), as well as determine their relative contribution to DOM mixture. Generally, red-shifted emission wavelengths (Em) represent the highly conjugated and aromatic nature of terrestrial plant compounds, whereas a shortening of wavelengths (i.e. blue-shift) is contrarily ascribed to the reduced-aromaticity of microbial compounds. The spectral extremes are moreover attributed to recalcitrant humic-like compounds versus recently produced labile peptides, with vegetal polyphenols falling somewhere in-between (Fellman et al., 2010; Hansen et al., 2016; Maie et al., 2008; Stedmon & Markager, 2005). Combining qualitative optical properties with quantitative measures (i.e. DOC) helps to reveal information about the scale and ecological significance of changes in the DOM pool. The joint analysis of quantity and quality is known as DOM composition.

Table 1.1 – Description of common DOM optical indices calculated from absorbance and fluorescence excitation (Ex) and emission (Em) spectra.

Adapted from Hansen et al. (2016).

Index	Calculation	Description
Biological index (BIX)	Em 380 nm divided by 430 nm, at Ex 320 nm.	Previously called the Freshness index. Recently produced microbial DOM versus recalcitrant vegetal DOM.
Humification index (HIX)	Peak area of Em 435-480 nm, divided by the peak area Em 300-345 nm + 435-480 nm, at Ex 254 nm.	Degree of humification and the presence of humic substances.
Fluorescence index (FI)	Ratio of Em 470:520 nm at Ex 370 nm.	Relative contribution of aromatic versus non-aromatic compounds.
Spectral slope ratio (S_R)	Non-linear fit to the absorbance spectrum of 275-295 nm divided by 350-400 nm.	Negatively correlated to molecular weight.

Table 1.2 – Description of common DOM fluorescent excitation (Ex) and emission (Em) peaks.

Adapted from Maie et al. (2008) and Stedmon & Markager (2005).

Peak	Ex / Em	Compound type	Source	Reactivity
A	< 260 / 448-480 nm	Humic-like	Vegetal	Recalcitrant
C	320-360 / 420-460 nm	Humic-like	Vegetal	Recalcitrant
M	< 250 / 370-430 nm	Humic- or fulvic-like	Microbial	Recalcitrant / Semi-labile
N/A	260-280 / 310-350 nm	Polyphenol-like	Vegetal	Semi-labile
B	270-275 / 304-312 nm	Protein-like (tyrosine)	Microbial	Labile
T	< 240 / 330-368 nm	Protein-like (tryptophan)	Microbial	Labile

SOM can be biochemically transformed via several different pathways, which in return influence its availability and position within biogeochemical cycles. Volatilization, microbial immobilization and physicochemical stabilization are three main pathways in which SOM can travel, respectively accounting for the loss, retention and protection of compounds. Incorporating

these pathways in the analysis of SOM, and the interpretation of its analyses, can lend a better understanding of how it is being redistributed throughout the environment.

(i) **Volatilization** – Numerous microbial metabolic pathways, mainly heterotrophic respiration, can transform SOM compounds into gaseous products such as CO_2 and NH_3. These byproducts are effectively volatilized and lost to the atmosphere, where they can be fixed back into vegetal and bacterial biomass. This volatilization leads to an immediate loss and depletion of SOM quantities. The transfer of organic matter from soil to air can be traced using stable isotopes. Microbes preferentially metabolize lighter and more abundant isotopes (e.g. ^{12}C, ^{14}N). These are incorporated into gaseous products and effectively removed from the soil upon volatilization. Heavier types (e.g. ^{13}C, ^{15}N) are then left behind in the biomass of soil microbes, which can be quantified using isotope-ratio mass spectrometry (IRMS). The addition of labile substrates has been shown to trigger soil isotopic enrichment by providing sufficient energy to fuel heightened respiration rates, and in return, volatilization (Gunnarsen et al., 2025).

(ii) **Microbial immobilization** – Microbes can also break-down SOM and utilize it as building-blocks for cellular components. This temporarily locks-up, or immobilizes, organic macromolecules within microbial biomass (Schmidt et al., 2011). This renders SOM inaccessible for plant uptake but otherwise allows it to be integrated within the food-web through microbivory (Coleman et al., 2018). It can moreover be re-released into the SOM pool upon cell death (i.e. necromass). The incorporation of SOM into microbial biomass is dependent on various factors, including but not limited to, environmental conditions, the availability of other supporting nutrients and the intrinsic growth rates of differing microbial taxa (Tang et al., 2024). Rates of bacterial biomass production (i.e. growth), as estimated from the incorporation of labelled [^{14}C] leucine, can provide a general idea of how much carbon is being immobilized within intracellular peptides (Bååth, 1994). These values can further be paired with bacterial respiration, to calculate (**Equation 1.1**) Bacterial Growth Efficiency (BGE), also known as Carbon Use Efficiency. The BGE value provides an estimate on the proportion of carbon being allocated towards growth over energy production (Giorgio & Cole, 1998). Shifts in BGE can therefore represent both

changes in bacterial metabolism and SOM cycling pathways (i.e. volatilization vs immobilization). A dip in BGE generally results from resource limitations, but it can also arise from environmental stress that pushes cells to allocate more energy towards maintenance and repair rather than growth (Condron et al., 2010; del Giorgio et al., 2011).

$$BGE\ (\%) = \frac{BP}{BP + BR} * 100$$

Equation 1.1 – Bacterial growth efficiency (BGE) calculated from the rates of bacterial biomass production (BP) and respiration (BR).

(iii) **Physicochemical stabilization** – As mentioned earlier, SOM compounds can become chemically and physically stabilized, thereby limiting their free-movement and access to microbial cells and enzymes. Most SOM stabilization occurs through the formation of MAOM from the adsorption of compounds to mineral surfaces via various interactions (e.g. cation bridges, ligand exchange, Van der Waals)(Kaiser & Guggenberger, 2000; Lützow et al., 2006). Adsorption occurs most in clay-rich soils, as clay's small particle size and crystalline structure imparts a higher surface area, and therefore a greater number of potential binding sites (Zhao et al., 2023). Stabilization can also occur through polymerization, similarly to how some HS are formed. Low molecular weight biomolecules, that would otherwise contribute to the labile SOM pool, can bind to one another via non-covalent interactions to form large supramolecular aggregates (Semenov et al., 2013). Under neutral or acidic conditions, aliphatic compounds can equally self-associate into micelle-like structures (de Melo et al., 2016). In both instances, enzymatic binding sites are occluded within the molecule's internal structure, thereby reducing its biodegradability. In addition, SOM can become physically occluded from microbial degradation if entrapped within soil particle aggregates (Marschner & Kalbitz, 2003). Fortunately, physicochemical stabilization is not permanent. Organo-mineral complexes and supramolecular aggregates can be disassembled following physiochemical changes in the soil environment like pH, redox potential, temperature, moisture and freeze-thaw cycles. Typical handling and preparation of soil samples (e.g. mixing, buffering) can easily

disrupt the interactions responsible for stabilization, thus making it difficult to capture and quantify its effect on SOM dynamics.

The interaction between SOM and microbes is not one-way. The transformation and cycling of SOM compounds may be dependent on microbial action, but SOM composition can moreover alter the very microbial communities and function that dictate its processing (Condron et al., 2010). Studies have demonstrated shifts in bacterial community diversity in response to changes in DOM quantity and quality. Soil communities normally dominated by rhizosphere associated species will gradually become populated by highly active zymogenous groups upon the addition of labile compounds (Wu et al., 2018). Such additions drive community changes by alleviating nutrient and energy restrictions of dormant species, while also eliciting competitive interactions over the newly available substrates (Bray et al., 2012).

SOM composition can moreover shift the function of soil microbes either through the metabolic potentials of emerging taxa or the plasticity of resident groups. Functional outcomes can be easily and effectively evaluated using community-level assays such as the redox-based technique of Biolog EcoPlates. Developed specifically for soil communities, these 96-well plates contain 31 carbon substrates that differ in chemical class and molecular complexity (carbohydrates, carboxylic acids, amino acids, amines, polymers, phenolic compounds) (Checcucci et al., 2021; Garland, 1997). An included tetrazolium dye creates a measurable (absorbance 590 nm) purple coloration upon a substrate's reduction by heterotrophic bacteria. A distinct substrate utilization pattern will emerge based on the metabolic capacities of the inoculated community. Depending on how the plates are analyzed, changes in these patterns can either represent shifts in community structure and/or metabolic potential. For instance, a greater functional propensity for degrading carbohydrate substates has been shown in communities that were previously exposed to high quality SOM, thus implying that SOM compounds can promote their own microbial breakdown (Schutter & Dick, 2001). Analyzing microbial community function through methods like Biolog EcoPlates™ is needed to reveal the bi-directional relationship between soil microbes and SOM. Without capturing function, we miss a huge controller of SOM cycling and ecological health.

Forensic taphonomy and the Cadaver Decomposition Island

Unlike the fields of ecology and soil biogeochemistry, forensic taphonomy is still in its infancy. The discipline only emerged four decades ago with the opening of the first HTF in Knoxville, Tennessee (Vidoli et al., 2017). At these facilities, donated human remains are typically surface deposited or buried for forensic and archeological research, teaching and training purposes. Taphonomic research also extends outside of HTFs through the use of human analogs, which allows researchers to perform studies across a wider range of geographical regions. Domestic pigs (*Sus scrofa*) are most commonly utilized due to their affordability, availability, and similarities to humans in anatomy, omnivorous diet and pilosity. Pigs also offer a greater level of replicability as individuals reared and slaughtered under the same conditions help to create a more homogenous sample group (Connor et al., 2018; Dautartas et al., 2018; Forbes, 2017). Analogs can provide valuable insights into decomposition processes, but there are limitations that must be considered. When compared to humans, pigs show dissimilarities in decay rates, decomposition byproducts, microbial processes and scavenging, potentially resulting from their greater enteric bacterial load and corporal fat content (Barton et al., 2020; Connor et al., 2018; Dautartas et al., 2018; DeBruyn et al., 2021; Knobel et al., 2019). It is therefore recommended that techniques and concepts developed using analogs be validated on cadavers prior to their application in human cases (Matuszewski et al., 2020)

One of the first concepts developed from animal- and human-based taphonomic research is the categorization of gross decomposition phenomena along a set of temporal phases (**Figure 1.1**). The first phase of "**Fresh**" begins moments after death. Most changes are microscopic, occurring at the cellular level with the onset of autolysis. The depletion of oxygen levels results in the dysregulation of intracellular enzymes, which ultimately attack and degrade cell membranes, thus leading to cell lysis (Gunn, 2019; Madea, 2023). The lack of oxygen, paired with the deactivation of the immune system, facilitates the rapid proliferation and transmigration of anaerobic gastrointestinal bacteria (Hyde et al., 2013; Janaway et al., 2009). The mortis triad is additionally present during this phase consisting of algor mortis (cooling of the body), livor mortis (internal pooling of the blood), and rigor mortis (stiffening of skeletal muscles) (Hayman & Oxenham, 2016). Putrefaction or the "**Bloat**" phase soon ensues where the fermentation and the

internal degradation of tissues by enteric bacteria leads to the production and accumulation of gases and fluids within the body cavity. This often leads a discolouration of the skin (e.g. purple, blue, green) and a characteristic distension or bloating of the abdominal region, neck and face. Drying, blistering and sloughing of the skin may also start to appear at this time, and continue to progress into subsequent phases (Emmons et al., 2021; Lee Goff, 2009). Eventually, the accumulated gaseous and liquid products will be purged from the body through orifices or tears in the skin. This purging marks the beginning of the "**Active**" phase and contributes to the formation of a CDI. In this phase, significant tissue loss will occur from microbial decomposers and colonizing necrophagous species (e.g. flies) (Carter et al., 2007). The body will then transition into the "**Advanced**" which is characterized by the exposure of underlying bones resulting from the depletion of soft tissues. The lack of easily consumable tissues will also lead to a drastic slowing in microbial and insect activity (Lee Goff, 2009). The final phase of "**Dry remains**" is achieved when only hard tissues (e.g. bone, desiccated skin, hair) and fatty residues remain (Forbes et al., 2017; Gunn, 2019). These five phases are not distinct but rather occur on a continuum (Lee Goff, 2009). The occurrence and/or duration of a given phase and its phenomena is subject to numerous intrinsic (i.e. within the body) and extrinsic (i.e. the environment) factors (Zhou & Byard, 2011).

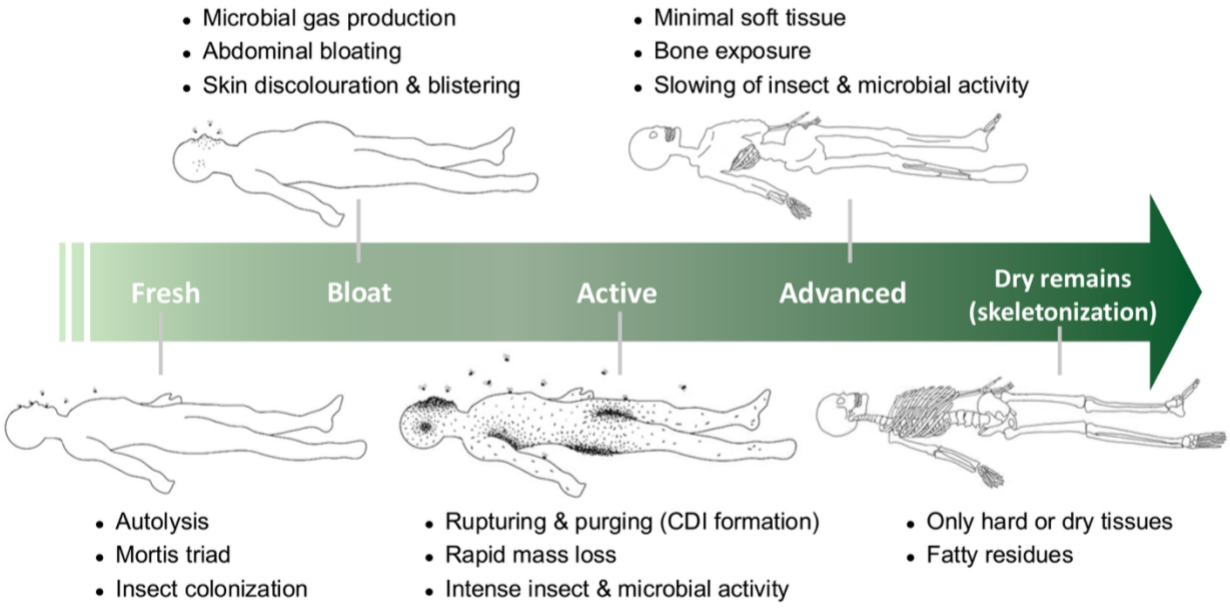

Figure 1.1 – The five phases of body decomposition and their main characteristics

Decomposing vertebrates have a unique body composition when compared to other forms of necromass (e.g. plant detritus). Animal tissues, particularly skeletal muscles, have a high nitrogen content, thus producing a very narrow C:N. Animal tissues are also rich in moisture, other key nutrients (e.g. P, S) and cations (e.g. K^{+}, Ca^{2+}, Mg^{2+}) (Carter et al., 2007). Moreover, the assorted diet of humans creates a distinct isotopic signature throughout the body's tissues. The dietary presence of animal products (i.e. dairy, eggs, meat) and C4 plants (e.g. sugar cane, maize) respectively enriches human tissues in both ^{15}N and ^{13}C (Tea et al., 2021). Contrarily to plants, vertebrate remains also carry a large and diverse gastrointestinal microbiome (Selber-Hnatiw et al., 2017). The distinct composition of animal and/or human bodies is translated to its purged products and the biochemistry of CDIs (Forbes et al., 2017).

The concentrated and localized leaching of vertebrate materials into surrounding soils leads to a visually distinguishable CDI that is dark in colouration and accompanied by dead vegetation; suffocated by the influx of viscous decomposition fluids (Larizza & Forbes, 2013). The spatial extent of a CDI is mostly determined by the body's mass, site of rupturing, maggot movement, precipitation and terrain characteristics like slope and soil texture. Displacement and disarticulation by vertebrate scavengers can additionally impact the shape and size of CDIs (Aitkenhead-Peterson et al., 2012; Carter et al., 2007; Luong et al., 2018). The center region of CDIs can be loaded with nitrogenous compounds (e.g. amino acids, $NH^{4+,}$ NO^{3-}) from both cadaver and microbial peptides (Keenan et al., 2018; Macdonald et al., 2014), fatty acids and sterols from adipose tissues (Larizza & Forbes, 2013; Luong et al., 2018), and ortho-phosphate from nucleotides, membrane phospholipids and osseous hydroxyapatite (Aitkenhead-Peterson et al., 2012; Dent et al., 2004; Fancher et al., 2017). CDIs can also be concentrated zones of DOC and total organic carbon (TOC), although increases are not always observed potentially due to rapid losses via bacterial respiration or bio-assimilation (Benninger et al., 2008; Damann et al., 2012). The increased concentration of macromolecules, in addition to bodily salts and minerals, produce rapid fluctuations in soil pH, electrical conductivity and oxidation potential (Carter et al., 2023). Decomposition products and their chemical effect within CDIs will dissipate with distance and time, but at varying degrees and rates depending on environmental, biologic and edaphic factors (Aitkenhead-Peterson et al., 2012; Carter & Tibbett, 2008a; Fancher et al., 2017).

Microbes are sensitive to environmental changes due to their high surface-to-area ratio and low ability to self-maintain homeostasis (Hermans et al., 2017). It is therefore unsurprising that the chemical disturbances described above are always followed by shifts in CDI soil microbiology. The increased availability of nutrient and energy substrates help to support the emergence of fast-growing and energetically demanding bacterial copiotrophs (i.e. Proteobacteria). Elevated respiration rates that are commonly observed in CDIs soils are most often attributed to the high energic demands of these taxa (Strickland & Wickings, 2015). Rapid bacterial O_2 consumption, along with the saturation of soil pores by decomposition fluids, collectively leads to the formation of hypoxic conditions (DeBruyn et al., 2024; Taylor et al., 2024). Low O_2 levels further encourage the presence of enteric anaerobic taxa (e.g. Firmicutes/Bacilli, Bacteroidetes, Clostridia) that are introduced upon the rupturing and purging of decomposition fluids (Carter et al., 2007; Cobaugh et al., 2015; Mason et al., 2023). The growing dominance of these groups is concomitant with a reduction in native aerobes and oligotrophs (e.g. Acidobacteria, Actinobacteria), who are poorly adapted to the shifting CDI conditions. The boom in CDI-specific bacterial groups will eventually subside as decomposition products are consumed, transformed and/or dissipated. This lends way to the gradual reestablishment of native taxa, but community diversity can still remain altered for a prolonged period of time (> 10 years) (Burcham et al., 2021; Strickland & Wickings, 2015). Advancements in next-generation sequencing technologies, particularly for the analysis of prokaryotic 16S rRNA gene sequences, has been central to uncovering the described successional patterns. The uniqueness of CDI condition and dynamics ensures that soil bacterial communities are consistently funneled through these patterns, which has made them a popular focus in the development of postmortem interval (PMI) estimation techniques (Finley et al., 2015; Metcalf, 2019).

Just as a decaying body influences soil biochemistry, environmental microbes can also colonize a body and influence the progression of decomposition. Bacteria emerging from the deposition substrate (i.e.: soil) or carried by necrophagous species are continuously populating a body throughout all phases of decay (Parkinson et al., 2009). Similarly to CDI soils, studies examining bacteria present on the surface of actively decomposing remains (i.e. epinecrotic microbiome) have also reported a rise in Proteobacteria. This taxon is largely unique to soils and

aquatic environments and is not predominant in vertebrate systems (Javan et al., 2016). Their presence in epinecrotic communities can therefore only be attributed to a soil-to-body transfer. Some evidence even suggests that the colonization by soil and environmental microbes contributes to, and potentially even enhances, tissue decomposition rates (Carter & Tibbett, 2008b; Lauber et al., 2014). As decomposition progresses into the later phases, a body's microbiome will increasingly resemble that of soils rather than the composition previously seen in the living or actively decaying host (Moitas et al., 2023).

Changing CDI community structures also bring about other functional and metabolic shifts, aside from heightened respiration. Research has demonstrated an enhancement in proteolytic, lipolytic and energy producing pathways within CDI bacteria (Burcham et al., 2024; Cobaugh et al., 2015; DeBruyn et al., 2021; Keenan et al., 2018; Mason et al., 2022; Singh et al., 2017). Functional differences between soil- and vertebrate-associated bacteria can moreover encourage cross-feeding and resource cycling within CDIs (Burcham et al., 2024; Keenan et al., 2023). These functional changes and partitioning are thought to collectively improve the transformation and turnover of decomposition products. Furthermore, repeated contact with decomposing vertebrates can potentially prime soil communities and elicit faster functional responses upon subsequent exposures (Carter & Tibbett, 2008b). Most of these ideas on CDI bacterial function and their outcomes are unfortunately speculative or inferential, as there is still a paucity in the literature. A more concrete understanding will only emerge with the continuation of function-based studies on CDI microbial communities.

HTFs strive to replicate natural conditions and forensically relevant scenarios, so that techniques and skills developed within these settings can be directly applied to real-world casework. But as outlined above, vertebrate decomposition drives an assortment of biochemical changes in CDI soils. The continual presence of decomposing remains within HTFs may therefore foster site-specific conditions, decomposition processes and biases that limit the broader transferability of research and training outcomes (Damann et al., 2012). In a review by Fiedler et al. (2023), they noted that many CDI studies tend to assess chemical and microbial shifts in isolation, often neglecting the mechanistic links between them. This significantly constrains our

capacity to gauge the severity of this issue and to eventually develop targeted strategies to negate it.

Taphonomic factors, seasonality and regionality

In addition to describing decomposition phases, early research in taphonomy was also concerned with identifying taphonomic factors; the set of biotic and abiotic factors that influence the decomposition and preservation of human remains. Mann et al. (1990) published one of the first studies on taphonomic factors, listing temperature, humidity/aridity and rainfall as some of the topmost influential variables at the Tennessee HTF. Follow-up studies noted how regional and seasonal variabilities in climate-related taphonomic factors create dissimilarities in decay rates (Archer, 2004; Bates & Wescott, 2016; Cockle & Bell, 2015; Galloway, 1996; Meyer et al., 2013; Parks, 2011; Suckling et al., 2016). Warm humid climates were concluded to accelerate decomposition by favouring microbial enzymatic activity, whereas arid sunlit conditions were seen to rapidly desiccate and preserve soft tissues (Galloway, 1996; Parmenter & MacMahon, 2009). Rainfall benefited decomposition by supporting the diffusion of microbes and substrates, but excess precipitation led to waterlogging and slower anerobic microbial processes (Archer, 2004; Carter et al., 2010). Due to the variability in taphonomic factors and their effects on decomposition, it became ill-advised to extrapolate taphonomic findings across biogeographic regions and seasons (Forbes, 2017; Giles et al., 2020). This realization prompted an expansion in global taphonomic research (human and animal) and the integration of environmental variables (e.g. accumulated degree days) within datasets. Despite this, winter and cold-climate conditions remained neglected due to the unsupported assumption that decomposition is negligible at < 0°C (Megyesi et al., 2005; Vass et al., 1992). With the lack of empirical data, most of our current understanding of sub-zero taphonomy stems from the examination of ancient human remains or frozen animal carcasses found respectively in glacial and arctic environments (Bereuter et al., 1997; Nývlt et al., 2016; Pilloud et al., 2016). Even less is known about the how the seasonal extremes (warm summer vs cold winter) of temperate continental climates impact taphonomy (Ribéreau-Gayon et al., 2023), let alone CDI formation and the microbial turnover of decomposition products.

Although microbial activity is greatly reduced in cold temperatures, soil-based evidence suggests that they can it still persist in below freezing conditions. The detection of CO_2, CH_4 and N_2O fluxes in frozen boreal and arctic soils has been regarded as indirect evidence of cold-temperature microbial activity (Öquist et al., 2009; Panikov, 2009). Furthermore, various incubation studies have recorded soil bacterial respiration, growth and enzyme synthesis between -4°C and -39°C (Drotz, Sparrman, Nilsson, et al., 2010; McMahon et al., 2009; Michaelson & Ping, 2003; Nikrad et al., 2016). From this evidence, it is not unreasonable to hypothesize that taphonomic and CDI related microbial activity can persist under cold conditions.

Various mechanisms can contribute to the maintenance of bacterial activity at low temperatures. Historical exposure to freezing can select for cold-tolerant environmental bacteria who are capable of synthesizing cryoprotectants, cold-shock RNA chaperones and substances responsible for maintaining membrane fluidity (e.g. fatty acids, carotenoids) (Chattopadhyay, 2006). Deep snow coverage can also have an insulator effect that decouples air temperature from that of the body and soil (Iancu et al., 2024; Kosolapova & Altshuler, 2024). The adsorption capacity of aromatic SOM compounds can moreover contribute to the formation of soil microaggregates and thin films of water along particle surfaces. In these films and small aggregates, the matric potential is greatly reduced, thus lowering the free-energy and freezing point of water. This helps to maintain the presence of liquid water which helps to minimize the damage caused to cells and aggregates by the expansion of ice and ensures the continued diffusion of microbes and substrates (Drotz, Sparrman, Nilsson, et al., 2010; Drotz, Sparrman, Schleucher, et al., 2010; Fagerlund, 1973; Spaans & Baker, 1996). Nevertheless, spring freeze-thaw cycles can still cause damage that leads to the release of SOM from unprotected cells and aggregates. The newly available SOM is then rapidly utilized by surviving microbes, consequently creating a characteristic spike in spring-time soil respiration (Feng et al., 2007; Skogland et al., 1988; Yanai et al., 2004). All these mechanisms that are well described in the soil sciences adds credibility to the idea that some taphonomic and CDI processes likely persist in cold climates and seasons, granted at a reduced rate and/or magnitude. Of course, this remains to be verified and compared to what occurs in warmer conditions.

Regionality shapes microbial processes in ways that extend beyond climate and seasonality. Edaphic features like pH, texture and parent material can influence how soil bacteria access and interact with resources (e.g. connectivity of pore spaces, adsorption) (Jeanbille et al., 2016; Nunan et al., 2017; Zheng et al., 2019). Regional differences in land use and historical disturbances (e.g. natural disasters) can moreover add spatial variability to selective pressures (Bissett et al., 2011; Labouyrie et al., 2023). Altogether, this creates regionally distinct filters on soil bacterial communities and their function. Soil from within the same climatic zone can therefore produce divergent microbial community structures, dissimilar responses to identical stimuli (e.g. amendments) and/or to variations in resistance and resiliency (Amarasinghe et al., 2024; Cui et al., 2023; Griffiths et al., 2008; Nunan et al., 2017; Zheng et al., 2019). When it comes to primarily vegetal inputs, this is known to create a regional heterogeneity in microbial-SOM dynamics (Doetterl et al., 2025). There is therefore a great precedence supporting the need to also examine CDI biochemistry and vertebrate-associated SOM across geographic regions.

Project goal and objectives

The concepts presented in the above literature review have been integrated into a single conceptual framework (**Figure 1.2**) that ties together vertebrate decomposition, SOM chemistry, bacterial responses and ecological health. Based on this framework, the overarching goal of the following dissertation was to evaluate the environmental impact of vertebrate decomposition by characterizing SOM-bacterial dynamics and relationships. This was achieved by examining in CDI soils:

1) The spatiotemporal changes in SOM source, quantity and lability (DOM optical properties, DOC levels, $^{13}C/^{15}N$ isotopic signatures).
2) The spatiotemporal changes in soil bacterial community structure (16S rRNA amplicon sequencing), metabolic activity (cell abundance, respiration) and metabolic function (biomass production, substrate utilization patterns).
3) The spatiotemporal patterns in SOM chemistry and bacterial responses across divergent temperate seasonal conditions (warm summer/spring vs cold autumn/winter) and biogeographic regions (Québec vs Hawaii).

4) The relationship between SOM chemical and bacterial variables.

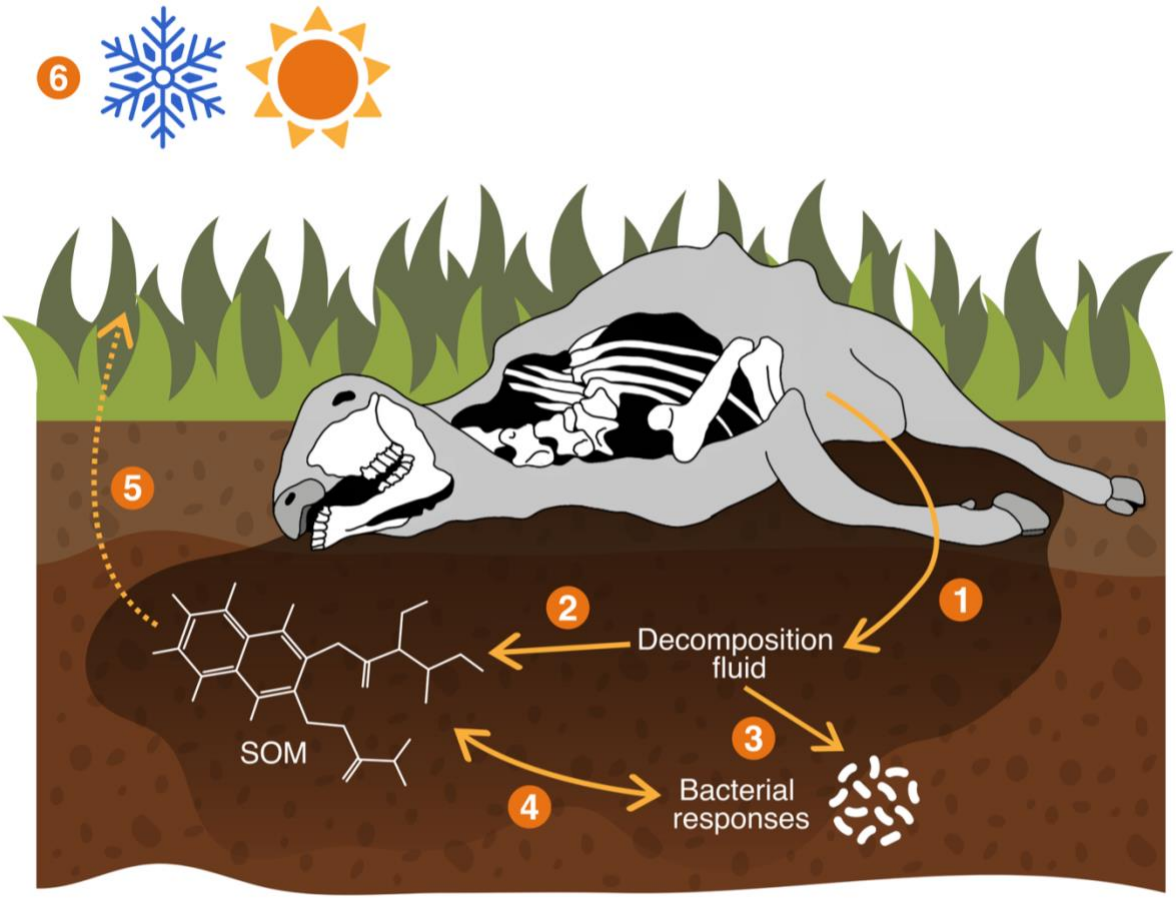

Figure 1.2 – Conceptual framework of doctoral dissertation

(**1**) Decomposing vertebrates release decomposition fluids into the surrounding soil, leading to the formation of a CDI. (**2**) Leached fluids may alter the chemical composition (quantity & quality) of SOM. (**3**) Leached fluids and altered SOM composition could then generate a shift in bacterial community structure. (**4**) The combination of 2 and 3 also creates shift in the metabolic responses (activity & function) of the soil bacterial community, which in return, influences the transformation and cycling of SOM. (**5**) Resulting SOM outcomes will either support or disrupt downstream ecological processes. (**6**) Taphonomy and SOM-bacterial dynamics can potentially differ across regions and season due to dissimilarities in climatic conditions and edaphic features. This dissertation addresses points 1-4 and 6.

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Chapter 2 – Organic matter composition as a driver of soil bacterial responses to pig carcass decomposition in a Canadian continental climate

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Abstract

Organic by-products are released into the surrounding soil during the terrestrial decomposition of animal remains. The affected area, known as the Cadaver Decomposition Island (CDI), can undergo biochemical changes that contribute to landscape heterogeneity. Soil bacteria are highly sensitive to labile inputs, but it is unknown how they respond to shifts in dissolved organic matter (DOM) quantity and quality resulting from animal decomposition. We aimed to evaluate the relationship between soil DOM composition and bacterial activity/function in CDIs under a Canadian temperate continental climate. This was studied in soils surrounding adult pig carcasses (n = 3) that were surface deposited within a mixed forested environment (Trois-Rivières, Québec) in June 2019. Using fluorescence spectroscopy and dissolved organic carbon analyses, we detected a pulse of labile protein-like DOM during the summer season (day 55). This was found to be an important driver of heightened soil bacterial respiration, cell abundance and potential carbohydrate metabolism. These bacterial disturbances persisted into the cooler autumn season (day 156) and led to the gradual transformation of labile DOM inputs into microbially sourced humic-like compounds. By the spring (day 324), DOM quantities and bacterial measures almost recovered, but DOM quality remained distinct from surrounding vegetal humic signals. All observed effects were spatially constrained to the topsoil (A-horizon) and within 20 cm laterally from the carcasses. These findings provide valuable insight into CDI organic matter cycling within a cold-climate ecosystem. Repeated CDI studies will however be required to capture the changing dynamics resulting from increasing global temperatures.

Keywords: Cadaver decomposition island, Dissolved organic matter, Soil biogeochemistry, Soil microbial ecology, Bacterial metabolism, Carrion.

Plain language summary

Soil bacteria are sensitive to environmental disturbances, but little is known on how they respond to chemical changes in organic matter resulting from terrestrial animal decomposition. We aimed to investigate over space and time the relationship between organic matter chemical characteristics and bacterial responses in soil impacted by animal decomposition in a Canadian temperate climate. This was achieved by sampling soil at increasing distances from adult pig

carcasses that were placed on the ground in a mixed forest of Québec. Large amounts of simple, easy to metabolize, protein-like compounds were found to be initially deposited into the topsoil close to the carcasses. These compounds helped to fuel changes in the metabolic activity and function of soil bacteria. Even over cooler seasons, bacteria continued to transform carcass inputs into more complex forms that remained chemically different from unaffected soils. This transformation decreased the presence of simple compounds, which helped to return bacterial responses close to normal conditions almost a year after carcass placement. This study will improve our understanding of how animal decomposition contributes to the cycling of organic matter in cold climates. Repeated studies will be needed to capture the effect of climate change on the observed soil trends.

Highlights

- Pig decomposition created prolonged and spatially constrained changes in soil DOM composition, as detected by fluorescence spectroscopy.
- Labile DOM inputs from carcasses drove shifts in soil bacterial respiration, abundance, and metabolic function.
- Microbial transformation of labile carcass inputs into humic-like compounds gradually occurred, even over cooler seasons.

Introduction

As an animal decomposes, a large quantity of fluid and organic compounds are leached into the surrounding soil following the autolytic, microbial, and larval breakdown of flesh. The biological by-products produced reflect the high protein, lipid and water content found in animal soft tissues, which differs from the composition of vegetation that is ubiquitously found in terrestrial environments (Achinewhu et al., 1995; de Lange et al., 2003; Ioan et al., 2017). As a result, the decomposition of an animal body leads to the formation of a biochemically distinct “hot spot” known as the cadaver decomposition island (CDI) (Carter et al., 2007). Affected soil experiences characteristic physiochemical changes (e.g., pH, moisture, redox potential, nutrient concentrations) that are often accompanied by shifts in microbial community composition, function, and activity (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017; Finley et al., 2016;

Pechal et al., 2013; B. Singh et al., 2018). CDIs are therefore considered a localized natural disturbance that contribute to soil heterogeneity (Carter et al., 2007). CDI properties are highly dynamic and can differ between decomposition phases, individuals, season, and soil type (Aitkenhead-Peterson et al., 2012; Breton et al., 2016; Stokes et al., 2009; Vass et al., 1992; Wilson et al., 2007). Semi-controlled CDI studies on animal or donated human remains have largely been directed toward forensic applications in post-mortem interval estimation and clandestine grave detection (Fiedler et al., 2023; Wescott, 2018). Such studies have also been principally conducted during the summer or in annually warm areas (e.g., Texas, Tennessee) because of the unverified assumption that microbially mediated decomposition ceases at below-freezing temperatures (Mason et al., 2023; Megyesi et al., 2005; S. Singh et al., 2024). In consequence, there is minimal empirical research that examines body decomposition within an ecological context, particularly in temperate regions (Barton, Cunningham, Lindenmayer, et al., 2013; Meyer et al., 2013). The ecological processes and seasonal effects that govern CDI biochemistry are hence largely unknown.

Carrion is a concentrated labile resource because of an animal's narrow C:N ratio, large mass and patchy distribution (Barton, Cunningham, Macdonald, et al., 2013; Carter et al., 2007). The drastic changes in soil conditions and nutrient availability accompanying animal decomposition tend to favor bacterial processes by promoting the proliferation of zymogenous and copiotroph bacterial groups (Carter et al., 2017; Forbes & Dadour, 2009). Levels of carcass-derived C and N are also more suited to supporting the stoichiometry of prokaryotes over fungi (Strickland & Rousk, 2010; Strickland & Wickings, 2015). Incubation studies have additionally demonstrated that soil bacterial communities are highly responsive to the addition of labile organic matter, and that they can subsequently affect its breakdown (Cleveland et al., 2007; de Graaff et al., 2010; Goldfarb et al., 2011; Lerch et al., 2011; Wardle et al., 2004). The functional response of dominant bacteria in CDIs can therefore be crucial in controlling the degradation and trophic transfer of labile carcass inputs. This can ultimately influence downstream consumers and overall soil productivity (Fiedler et al., 2023). However, the dynamics between soil bacteria and organic matter have been minimally evaluated under the unique scenario of animal decomposition. This has left a gap in the mechanistic understanding of how and when carrion-

derived organic matter is cycled in terrestrial ecosystems. Such information is needed to better establish the role of dead animal biomass in ecosystem food-webs and nutrient/energy budgets (Barton, 2015; Barton & Bump, 2019).

Dissolved organic matter (DOM) is the soluble and most bioavailable fraction of organic matter in soils. The degree of DOM's influence on soil bacteria and its cycling is partly dictated by its chemical composition, which is characterized by its quantity and quality (Chapin et al., 2002; Chenu et al., 2014). Soil DOM quantity is commonly evaluated by using organic carbon concentrations as a proxy, given that DOM can be composed of up to 50% of carbon in mass (Strawn et al., 2015). DOM quantity provides insight into the flux of organic material, but the measure fails to inform about lability, transformation, and cycling. This can be addressed through the characterization of DOM quality. Small, simple compounds generated from biological activity (e.g., amino acids from metabolites) are deemed high-quality, for they can be readily transformed by microbial enzymes. Large, conjugated, and aromatic compounds originating from plant matter (e.g., lignin, tannin) or polymerization (i.e., humic substances) are contrarily low-quality and recalcitrant because of the greater degree of microbial specialization needed for their metabolism (Chapin et al., 2002; Marín-Spiotta et al., 2014; Strawn et al., 2015). DOM quality can be inferred using cost-effective spectrofluorometric techniques and statistical modelling. This is possible because of an optically active fraction of DOM compounds that can produce three-dimensional excitation-emission (EEM) spectra based on their molecular structure (Fellman et al., 2010; Hansen et al., 2016; Stedmon et al., 2003). Significant shifts in DOM composition can result from the introduction of new sources, microbial processes and humification. Hence, fluorescence spectroscopy has been used in combination with quantitative analyses to track DOM inputs and their transformation in various natural and engineered systems (D'Andrilli et al., 2019; Ishii & Boyer, 2012). Characterising DOM composition in CDI soils can similarly provide insight on carrion's value to microbial communities and its contribution to labile and recalcitrant pools.

The following study aimed to evaluate the spatiotemporal relationship between soil DOM composition and bacterial responses to CDI formation under a Canadian temperate continental climate. It was hypothesized that the decomposition of animal remains would introduce a large quantity of DOM and shift chemical characteristics from vegetal-dominated humic substances to

labile microbially sourced protein-like compounds. This was moreover expected to fuel increases in bacterial activity and promote functional changes that support the degradation of high-quality substrates. Changes were anticipated to be greatest following the complete purging of fluids and were assumed to decrease over time from the gradual transformation and depletion of labile carcass-derived material. This was tested over three seasons (summer, autumn, spring) by examining the EEM spectra, DOC concentration and different aspects of bacterial metabolism in soils surrounding pig carcasses placed in a forested site of Trois-Rivières, Quebec. Labile carcass DOM was initially found to stimulate bacterial metabolic responses in nearby soil. This was followed, during the cooler seasons, by the gradual microbial humification of DOM and eventual restoration of soil bacterial function.

Materials and methods

Experimental site and set-up

The carcasses of three domestic adult pigs (Sus scrofa domesticus Erxleben), weighing approximately 90 kg each, were obtained the morning of 18 June 2019, from the Boucherie Côté at Sainte-Eulalie (Quebec, Canada). The pigs were killed with a perforating gun in accordance with Section 12 of the Quebec Animal Welfare and Safety Act, and the use of pigs was authorized under Section 11.1 of the Quebec Food Products Act. Each carcass was placed in a plastic body bag and transported to a 100 m^2 experimental site located on the campus of the Université du Québec à Trois-Rivières (46.3446°N, −72.5834°W). The carcasses were surface deposited directly on the soil with a minimum of 10 m between each carcass. When not sampling or conducting observations, galvanized mesh cages were placed over the carcasses to prevent scavenging and the dispersion of remains by large animals.

The experimental site was situated in a temperate mixed forest within the Saint-Laurent lowlands ecoregion (Jobin et al., 2010). The area was populated by white pine (*Pinus strobus*), red maple (*Acer rubrum*), white spruce (*Picea glauca*) and American beech (*Fagus grandifolia*). The forest foliage created a closed canopy with an open understory, which provided a mix of sunny and shaded areas throughout the day (Maisonhaute & Forbes, 2020). The terrain had a minor slope (0%–3%) and consisted of an imperfectly drained humo-ferric podzolic soil with a sandy

texture. The soil profile in the area on average had an A-horizon depth of 0–14 cm and B-horizon depth of 14–50 cm (Soil Landscapes of Canada Working Group, 2010). The climate of the region is classified as snowy, fully humid with warm summers (Dfb) according to the Köppen-Geiger classification system (Peel et al., 2007).

Sampling

Soil samples were collected laterally from the carcass at increasing distances uphill (-20, -50 cm) and downhill (20, 50, 100, 200 cm) using a manual slide hammer soil corer (7.62 × 15.24 cm). 0 cm samples were taken as close as possible to the trunk or abdomen of the carcass. An uphill and downhill control sample was also obtained from outside the perimeter of the experimental site (**Figure S2.1**). Soil cores were wrapped in aluminum foil and transported by cooler to be stored at 4°C before treatment. Sampling for the summer, autumn and spring seasons were respectively conducted on day 55, 156 and 324. During this period, the carcasses remained in a differential decomposition state of advanced decay and dry remains. Carcass skin was dry and leathery in appearance with partial bone exposure and accompanied by the presence of adipose tissue and lipid residues that were colonized by cheese skipper larvae (Piophilidae) (Maisonhaute & Forbes, 2020). No winter samples were collected because of deep snow cover and ground frost.

Soil and slurry preparation

Soil cores were processed by discarding the organic layer and carefully dividing the soil of the A-horizon (topsoil) and B-horizon (subsoil) into separate aluminum dishes. The soil was placed under a fume hood to airdry at ~20°C for 1 week. Once dried, the soil was sieved (2 mm) to remove debris and break apart aggregates. The dried and sieved soil was then stored in sealed plastic bags at 4°C until slurry preparation.

A 1g sample of dried-sieved soil was mixed with 40 mL ultrapure water and shaken overnight at 4°C. Soil particles were allowed to settle for a minimum of 30 min, to which the supernatant was extracted and buffered to 0.001 N $NaHCO_3$ to replicate the ionic strength of natural soil systems (Wickland et al., 2007). The buffered slurry was then passed through pre-combusted (500°C, 4 hr) GF/F (0.7 μm) or GF/D (2.7 μm) glass microfiber filters, respectively, for

chemical and bacterial analyses. A subset of air-dried soil was further dried at 105°C for 24 hr in order to determine the residual moisture content to correct absolute values into units of dried-weight soil.

Chemical analyses

For DOM quantity, the concentration of dissolved organic carbon (DOC) in acidified (HCl, pH < 2) GF/F slurries were measured using a Sievers M9 Portable TIC/TOC Analyzer (GE Analytical Instruments, Boulder, CO). The instrument was verified against a five-point potassium hydrogen phthalate calibration curve and samples were run in triplicates with an accepted relative standard deviation of ≤10%.

DOM chemical characteristics were evaluated based on the absorbance and fluorescence EEM spectra of GF/F slurries. Fluorescent EEMs were created using a 1 cm glass cuvette and a Carey Eclipse Fluorescence Spectrometer (Agilent, Santa Clara, CA). EEMs were collected at excitation wavelengths (Ex) of 230–540 nm and emission wavelengths (Em) of 300–600 nm respectively at 5 and 2 nm intervals. Instrument biases were removed using manufacturer-provided correction files. Fluorescent spectra were corrected for inner filter effects by using the absorbance spectra (200–800 nm) of samples analyzed in a 1 cm quartz cuvette and a Carey 100 UV-Vis Spectrophotometer (Agilent, Santa Clara, CA). EEMs were normalized to Raman units (R.U., nm^{-1}) using the area under the curve of the Raman scatter peak of ultrapure water. Fluorescent and absorbance based spectral indices were obtained using the ratios and calculations described in Fellman *et al*. (2010). The freshness index (BIX) served as an indicator of recently produced microbial DOM versus highly decomposed senescent DOM (Huguet et al., 2009). The humification index (HIX) designated the degree of DOM humification, which is associated with structural complexity (Ohno, 2002; Zsolnay et al., 1999). Microbial and terrestrial DOM sources were identified using the fluorescent index (FI) (Cory & McKnight, 2005). Lastly, DOM molecular weight was represented by the spectral slope ratio (S_R) using the absorbance slope of 275–295 nm/350–400 nm (Helms et al., 2008). A total of 133 EEMs were collected in this study and analyzed using Parallel Factor Analysis (PARAFAC) as described by Murphy *et al*. (2013). PARAFAC is a multivariate modeling technique that decomposes 3D EEMs into independent

fluorescent signals that are indicative of DOM chemical composition and source. The analysis was run using the drEEM toolbox 0.6.3 for MATLAB 2021b (Mathworks, Natick, MA, USA).

A four component (C1-C4) PARAFAC model with 99% explained variation was validated using split-half analysis (**Figure S2.2**). The model was uploaded to the OpenFluor online database (Murphy et al., 2014) for component identification. A search with a similarity score of 92% yielded matches for all four components. C1 (Ex_{max} < 250, Em_{max} 458 nm) matched with components associated with humic-like substances of terrestrial origin (Murphy et al., 2011). C2 (Ex_{max} < 250, Em_{max} 414 nm) matched as a lower molecular weight humic- and/or fulvic-like component that is linked to autochthonous microbial activity (Garcia et al., 2018; Murphy et al., 2011). C3 (Ex_{max} 280, Em_{max} 336 nm) corresponded to protein-like compounds that resemble free or bound tyrosine and tryptophan resulting from recent microbial peptide degradation (Fellman et al., 2010; Lapierre & del Giorgio, 2014; Wheeler et al., 2017). Matches for C4 (Ex_{max} < 240, Em_{max} 312/364 nm) reported confounding identifications. The component was described as humic-like (Dainard et al., 2019), protein-like (Bittar et al., 2016; Dainard et al., 2019; Lapierre & del Giorgio, 2014; Wheeler et al., 2017), quinone-like (Cory & McKnight, 2005) or inconclusive (Jørgensen et al., 2011; Murphy et al., 2018). This disagreement may be because of an overlap between the fluorescent signal of humic phenols (e.g., tannins) and the phenolic hydroxyl of labile tyrosine (Maie et al., 2007). The production of quinones from the oxidation of phenol-containing lignin likely further obscured signal identification (Hernes et al., 2009). The overlap between compounds would explain for the double emission maxima reported by the model. For simplicity, C4 was decided to be identified as a phenol-like component of terrestrial and/or microbial origin. Component values were calculated and analyzed as the precent contribution (%C1–%C4) to the maximum fluorescent intensity (F_{max}, R.U.).

Bacterial analyses

Rates of bacterial respiration (BR) of GF/D slurries were measured in the dark at 20°C using 4 mL glass vials equipped with PSt5 optical oxygen sensors and a SDR SensorDish™ (PreSens, Regensburg, Germany). Readings were automatically taken every 60 min for a maximum of 2 weeks or until respiration rates plateaued. The rate of oxygen consumption was obtained from

the slope of the linear regression line of O_2 ($mg \cdot L^{-1}$) versus time. The rate of O_2 respiration was converted to the rate of carbon consumption using a respiratory quotient of 0.95 (Hilman et al., 2022).

For bacterial cell abundance (BA), 1.5 mL of GF/D slurries were fixed to a final concentration of 0.5% glutaraldehyde and stored at −80°C until analysis. Thawed bacterial cells were stained with 50X SYBR™ Green (Invitrogen, Walham, MA) and incubated at room temperature in the dark for 15 min. Cells were counted on a BD Accuri™ C6 Flow Cytometer (BD Biosciences, Franklin Lakes, NJ) with an acquisition threshold of 10,000 events. Samples additionally filtered through GF/HP (0.22 µm) were stained and ran as blanks for a limit of 1 min. TruCount™ (BD Biosciences, Franklin Lakes, NJ) fluorescent beads (24.3 $beads \cdot \mu L^{-1}$) were used to determine the true flow rate. Data processing was done on BD CSampler™ Plus software (BD Biosciences, Franklin Lakes, NJ). Absolute cell counts were obtained from the log-scale of FL1 (green, 533 nm) versus FL3 (red, >670 nm) density plots, and electronic gates were determined based on the strategy described by Hammes and Egli (2010). Biolog EcoPlates™ (BioLog Inc., Hayward, CA) were used as an in vitro technique to assess the functional response of heterotrophic bacteria in our sampled soil. This technique worked by quantitively measuring the reduction rate of 31 sole carbon substrates (**Table S2.1**) that are known to have high discriminatory power in soil bacterial communities (Hitzl et al., 1997). Substrate utilization patterns were used as an indicator of metabolic potential (Stefanowicz, 2006). 120 µL of GF/D slurry was pipetted into the wells and stored in the dark at room temperature. Purple coloration from substrate reduction was optically measured at an absorbance of 590 nm using a Synergy H1 microplate reader (Agilent, Santa Clara, CA). Absorbance measurements were taken twice daily for the first week, and once daily for the second week until the average plate well color development (AWCD) plateaued. The AWCD of individual blank corrected substrates were normalized to a plate AWCD of 0.5 to account for differences in well inoculum density (Garland, 1997; Garland & Mills, 1991). Negative values for normalized substrate AWCD were considered as zero.

Statistics

All statistical analyses and graph building were carried out in RStudio version 4.2.1 (R Core Team, 2023). A permutational multivariate analysis of variance (PERMANOVA, with 999 permutations) was performed using *vegan* 2.6–2 (Oksanen et al., 2022) to evaluate global difference between soil horizons. The test was carried out using Euclidean distances with pigs as a blocking factor to account for inter-individual variability. Seasonal and spatial differences in DOC, BR and BA were tested via linear mixed effects model with pigs set as a random effect using *lme4* 1.1.30 (Bates et al., 2015). This was followed by a two-sided Dunnett's test with Bonferroni adjustment via *multcomp* 1.4–20 (Hothorn et al., 2008). Principal component analysis (PCA) was generated using *stat*s 4.2.1 (R Core Team, 2023) to reveal patterns in DOM composition (DOC, spectral indices, PARAFAC components) along sampling distance and season. A PCA was similarly performed on the normalized AWCD of each Biolog EcoPlate™ substrate to examine spatial and temporal trends in bacterial metabolic potential. Partial least squares regression (PLSR) with leave-one-out cross-validation was performed with *plsdepot (Sanchez, 2012)* to evaluate if the x-variables of DOM composition (DOC, PARAFAC components, spectral indices) drove changes in the y-variables of bacterial activity/function (BR, BA, metabolic potential). A linear regression, *stats* 4.2.1 (R Core Team, 2023), between the PLSR scores of t1 and u1 was additionally conducted to view the correlation between *x* and *y* variables over sampling distances and season. All multivariate analyses were performed on centered and scaled data. A significance value of 0.05 was adopted across all statistical analyses.

Results

Vertical extent

A PERMANOVA was performed on the entirety of chemical and bacterial measures to evaluate if universal differences occurred within the A- and B-horizons of the sampled soil. This was done to determine the vertical extent of the CDI's effect. Results indicated that the A-horizon differed across both sampling distances (Df = 6, F = 1.463, p = 0.001) and season (Df = 2. F = 3.47, p = 0.001), for chemical and bacterial measures altogether. Dissimilarly, no differences were detected across sampling distance in the B-horizon (Df = 6, F = 1.02, p = 0.326),

but measures did demonstrate significant seasonal changes (Df = 2, F = 4.72, p = 0.001). Mean values for DOC, BR and BA were generally lower for the B-horizon when compared to the A-horizon (**Figure S2.3**). It was therefore concluded that the vertical extent of carcass decomposition did not exceed the A-horizon, since the B-horizon remained stable across all lateral distances. Only naturally occurring seasonal shifts in DOM and bacteria were measurable within the subsoil. For this reason, all subsequent results are focused only on the A-horizon.

DOM composition

Linear mixed effect model revealed significant differences in DOC concentration within the A-horizon across distance (Df = 7, F = 3.06, p = 0.015) and season (Df = 2, F = 7.66, p = 0.001). DOC in soil at -20 and 0 cm from the pig carcasses underwent the greatest degree of change in the summer, with a respective 266% and 368.16% increase when compared to the controls. These concentrations decreased over time but remained slightly elevated by the spring at an increase of 161% and 198% (**Figure 2.1A**).

The percent contribution of each PARAFAC component to total fluorescence varied greatly across distance and season. C1 generally remained stable over all sampling distances and seasons, for it represented plant-derived humic substances that are universally present in a forested setting (**Figure 2.1B**). The greatest peak in C2 was observed at point -20 cm, with an increase from the control of 31%, 10% and 13% respectively for the summer, autumn, and spring seasons (**Figure 2.1C**). The opposite effect occurred for C4, where component contribution declined the most at −20 cm, resulting in a decrease of -79%, -49% and -85% across the three seasons (**Figure 2.1E**). The majority of sampling distances during the summer experienced a decrease in C3 contribution. This was because of unusually high C3 values obtained for the 200 cm and the control samples. Excluding the 200 cm and the controls, the next greatest peak in C3 for the summer was observed at 20 cm, which was 67% greater than the lowest value recorded at -50 cm. The greatest peak in C3 for the proceeding autumn and spring was observed at 0 cm, with respective increases of 160% and 154% from the controls (**Figure 2.1D**).

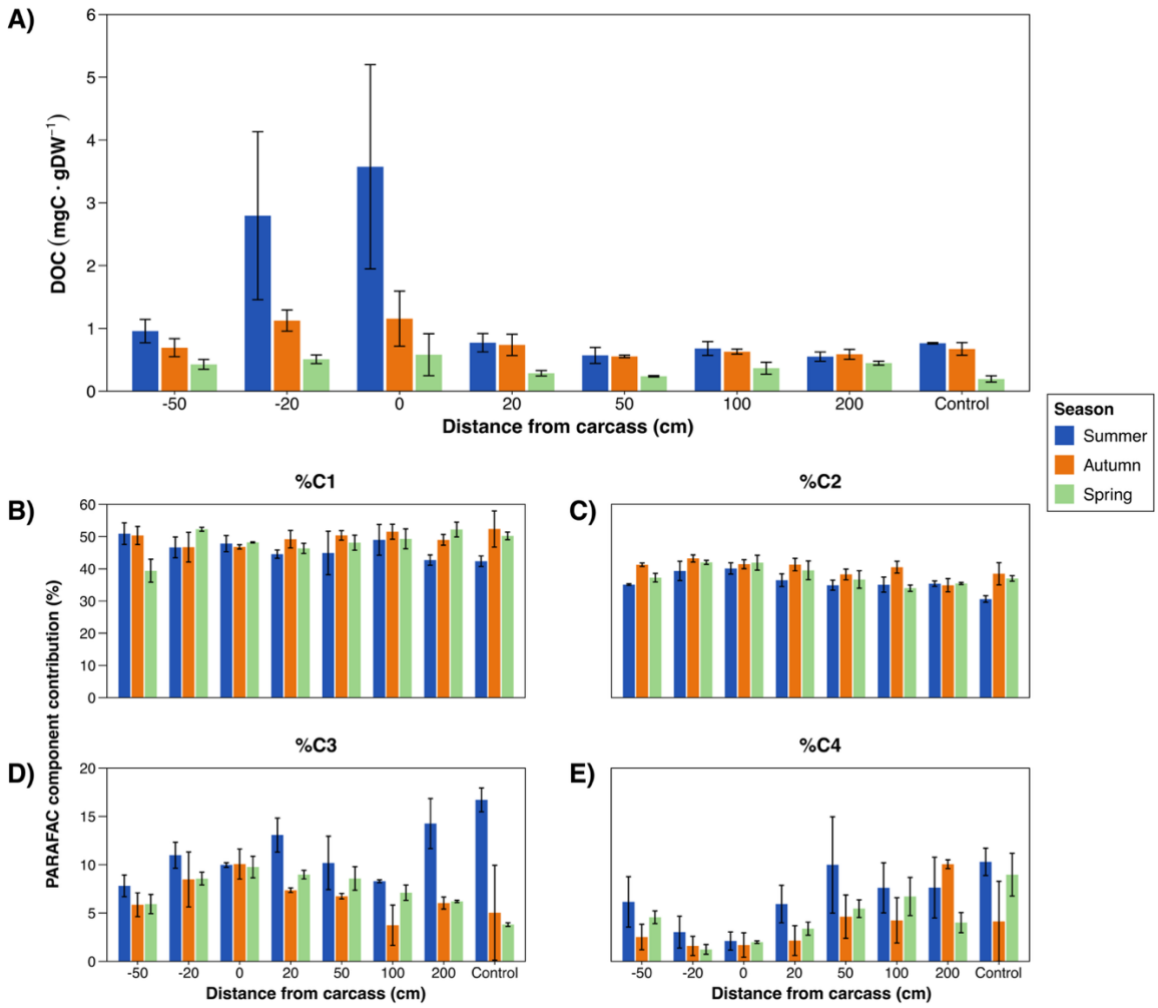

Figure 2.1 – Seasonal trends in soil (A-horizon) DOC and PARAFAC components around decomposing pig carcasses.

Mean and standard error of DOC concentration (**A**) and the relative percent contribution of PARAFAC components C1 (**B**), C2 (**C**), C3 (**D**) and C4 (**E**) from soils sampled at varying lateral distances. Control samples taken outside of the experimental site are included as the furthest distance from the carcass.

A principal component analysis (PCA) of relative PARAFAC component contribution (%C1–%C4), spectral indices (BIX, HIX, FI, S_R) and DOC concentration demonstrated a shift in DOM composition across season and sampling distance (**Figure 2.2**). PC1 (35.4% explained variation) represented naturally occurring seasonal shifts, whereas PC2 (25.3% explained variation) distinguished the effect of carcass decomposition. Spring and autumn samples were similarly

driven toward the right quadrants by the positive loadings of %C1, FI and HIX along PC1, whereas summer differed toward the left by the negative loading of %C3. Summer sampling distances closest to the carcasses (-20 and 0 cm) were strongly driven downwards by the negative loadings of DOC and BIX along PC2. Autumn and spring samples at -20, 0 and 20 cm were similarly driven by the negative loading of DOC but also appeared strongly influenced by the negative loading of %C2 on PC2. All other sampling distances (-50, 50, 100, 200 cm) and controls across the three seasons were contrarily shifted toward the upper quadrants by the positive loading of %C4 along PC2. Furthermore, the small loading for SR suggested that strong variations in molecular size did not occur across season and sampling distance.

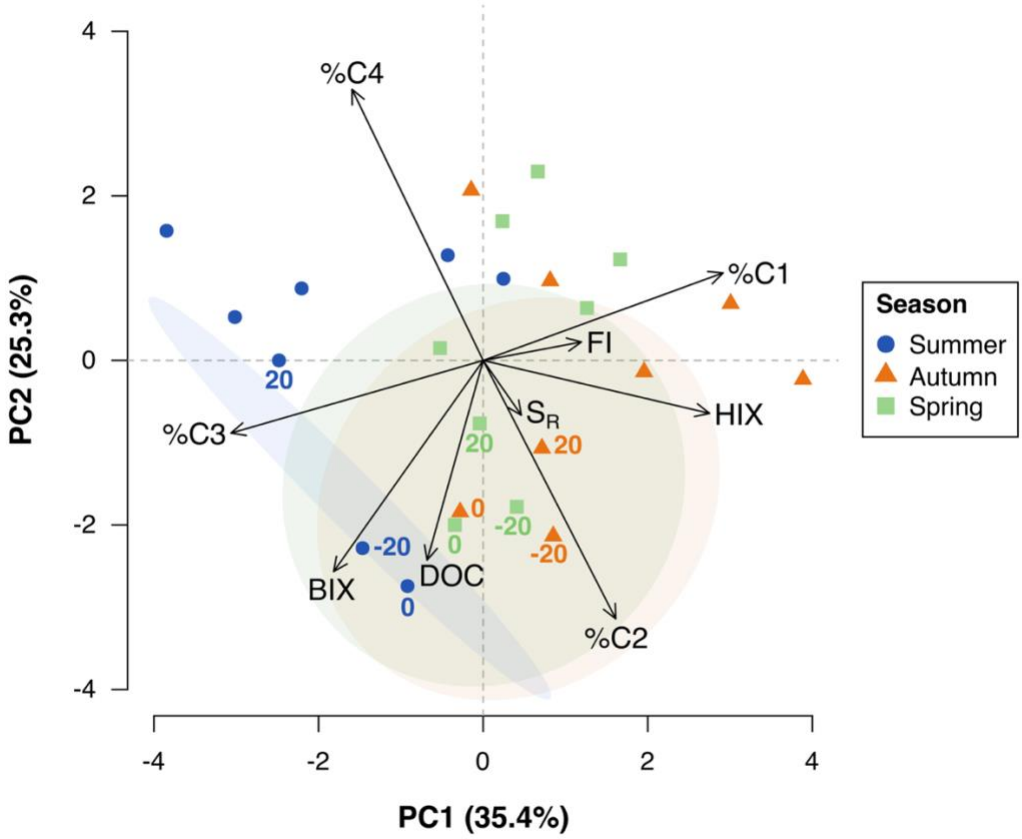

Figure 2.2 – Seasonal trends in soil (A-horizon) DOM composition around decomposing pig carcasses.

PCA biplot of measures for DOM quantity (DOC) and quality (%C1-%C4, BIX, HIX, FI, SR) obtained from soils sampled at varying lateral distances. PCA includes 95% confidence ellipses for labeled points that were sampled closest to the carcasses (−20, 0 and 20 cm).

Bacterial activity and function

Mean rates of bacterial carbon respiration (BR) significantly differed across sampling distance (Df = 7, F = 2.95, p = 0.031) but not season according to a linear mixed effects model. Temporal trends were however noticeable, particularly for soil sampled closest to the carcasses (**Figure 2.3A**). At 0 cm, BR increased by 500% from control values during the summer season. Respiration rates remained at an increase of 400% in the autumn and decreased slightly below controls at -11% in the spring.

Mean cell counts for bacterial abundance (BA) similarly differed across distance (Df = 7, F = 3.51, p = 0.002). Although no significant differences were detected across season, clear temporal trends also emerged for samples taken at -20 and 0 cm (**Figure 2.3B**). These samples respectively experienced a summer increase of 628%, and 285% from the controls. Absolute bacterial cell concentrations at these distances gradually decreased over time but still remained elevated relative to the controls at an increase of 1,736% and 1,238% in the autumn, and 1,619% and 1,248% in the spring.

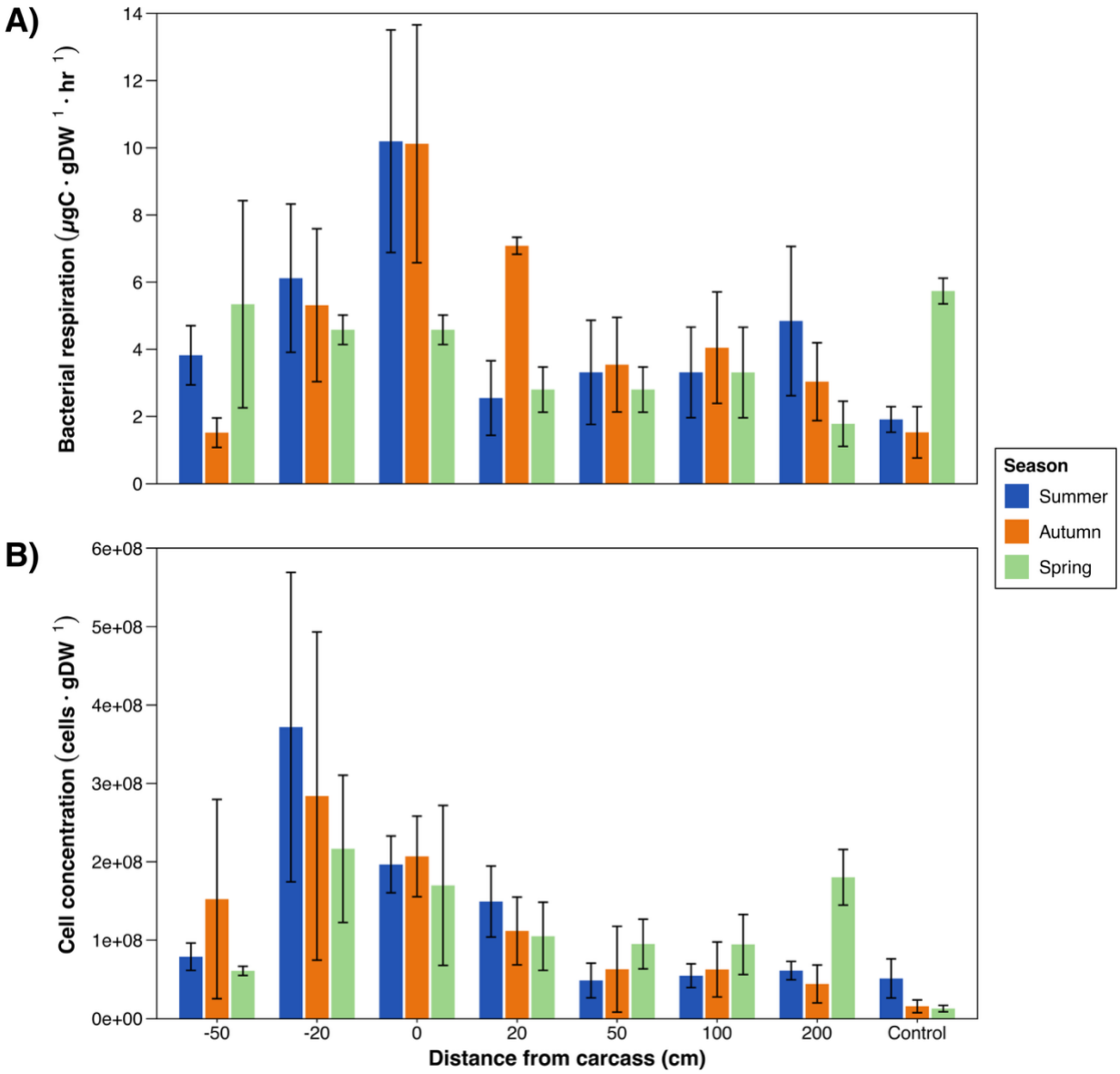

Figure 2.3 – Seasonal trends in soil (A-horizon) bacterial abundance and activity around decomposing pig carcasses.

Mean and standard error of bacterial carbon respiration (**A**) and cell abundance (**B**) from soils sampled at varying lateral distances. Control samples taken outside of the experimental site are included as the furthest distance from the carcass.

Changes in bacterial metabolic potential were assessed using Biolog EcoPlates™. A PCA (**Figure 2.4**) of average well color development (AWCD) demonstrated a shift in substrate utilization patterns across season and sampling distance. Seasonal effects were mostly distinguished by PC2 (14.7% explained variation), with autumn and spring falling within the lower quadrants, and summer within the upper quadrants. Decomposition driven shifts in metabolic potential appeared along both PC1 (27.4% explained variation) and PC2. Summer samples at −20, 0 and 20 cm were distinct from all other distances and appeared to be strongly driven by the negative PC2 loadings of several carbohydrate substrates (C3, C4, C7, C8, C10) and Phenylethyl-amine (A1). In addition to PC2, the positive PC1 loadings for the same substrates contributed to a shift in -20, 0 and 20 cm autumn samples. Pig carcass decomposition therefore shifted scores toward the lower right quadrant of the PCA. Metabolic potential for the remaining autumn (-50, 50, 100, 200 cm and controls) and spring samples were notably influenced by the negative loadings of carboxylic acids (CA2, CA3, CA4, CA5) along PC1. The lack of separation for points -20, 0 and 20 cm from further sampling distances in the spring suggested a return to baseline seasonal substrate utilization patterns.

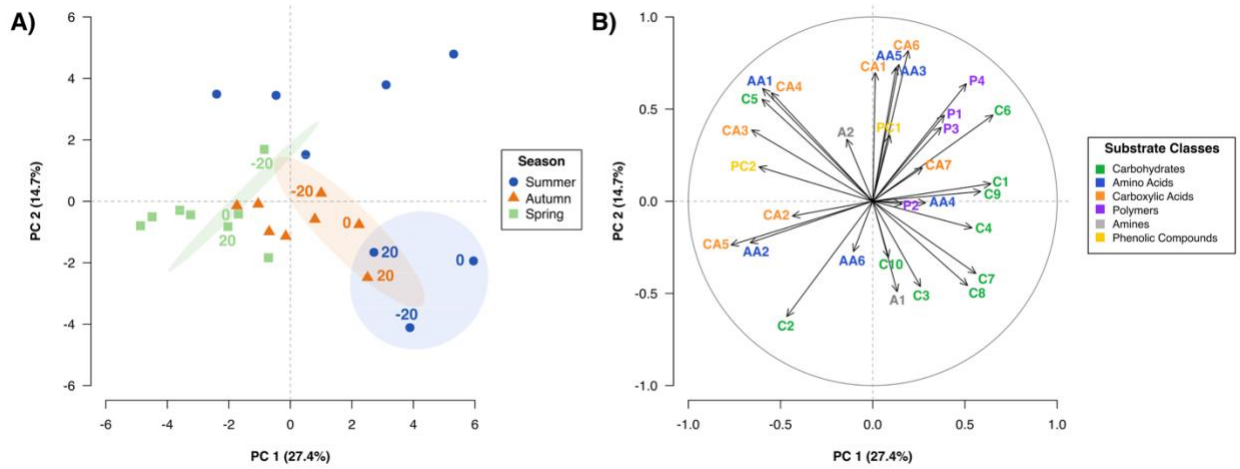

Figure 2.4 – Seasonal patterns in soil (A-horizon) bacterial metabolic potential around decomposing pig carcasses.

PCA score (**A**) and loading (**B**) plots of Biolog EcoPlate™ substrate color development generated from bacteria collected from soils sampled at varying lateral distances. Substrates are categorized into their respective chemical classes: carbohydrates (green C1-C10), amino acids (blue AA1-AA6), carboxylic acids (orange CA1-CA7), polymers (purple P1-P4), amines (gray A1-A2) and phenolic compounds (yellow PC1-PC2). PCA score biplot includes 95% confidence ellipses for the labeled points sampled closest to the carcasses (−20, 0, 20 cm).

Influence of DOM composition on bacterial responses

Partial least squares regression (PLS-R) was performed to evaluate the influence of carcass related DOM compositional changes on bacterial function and activity (**Figure 2.5A**). First two components of the model explained 59.5% of the total variation. Bacterial respiration, cell abundance and the metabolism of carbohydrate substrates C3, C7 and C8 were highly correlated with DOC concentration. These bacterial response variables were also positively correlated to BIX and %C2, but to a lesser extent. BIX was however strongly correlated with an increased metabolic potential for C1, C4, C6, C9, and CA7. %C2 was moreover closely related to A1 and AA4 substrate reduction. A linear regression of the x-scores (t1) and y-scores (u1) from the first component of the PLS-R model demonstrated a positive linear relationship (R^2 = 0.632) between the model scores for DOM composition and bacterial responses (**Figure 2.5B**). Samples were uniquely distributed along the linear regression based on season and distance; with further distances (−50,

50, 100, 200 cm, controls) in the autumn and spring visibly clustering toward the lower left of the plot, versus proximal (-20, 0, 20 cm) samples in the summer extending more toward the upper right. DOC concentration, BIX and % C3 were identified as the top three most influential predictor variables of bacterial responses, as seen in the variables of importance to prediction (VIP) scores (**Figures 2.5C** and **2.5D**).

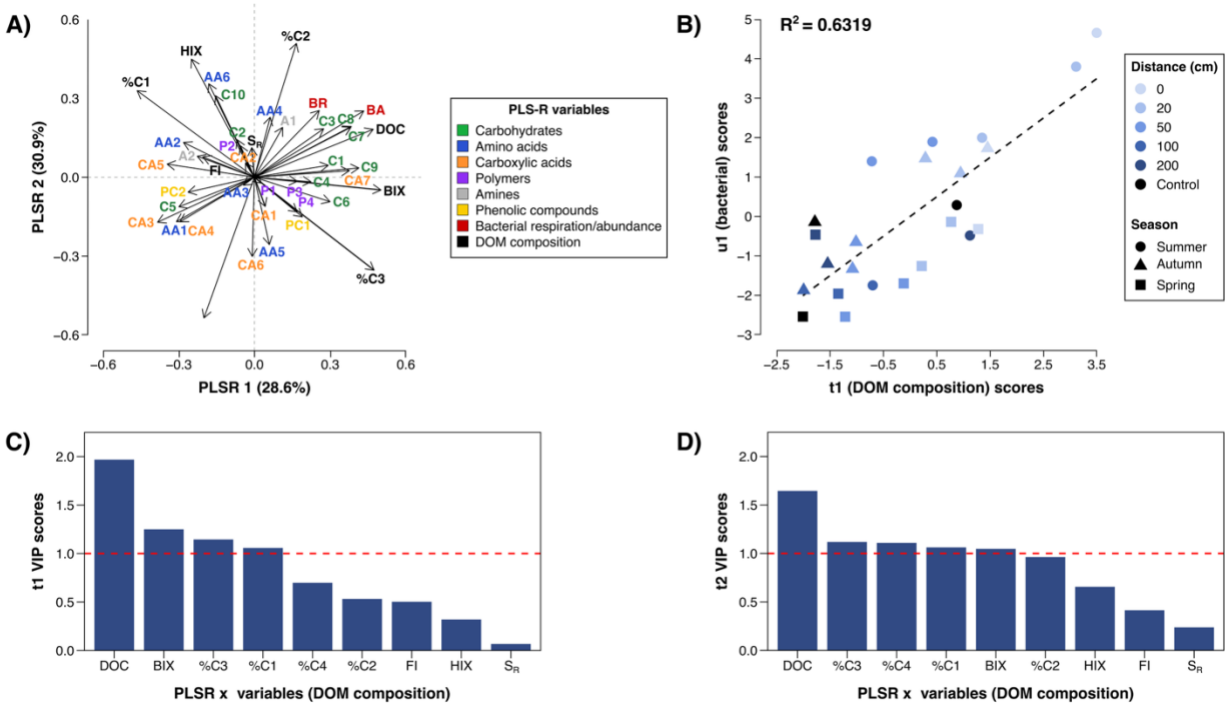

Figure 2.5 – Relationship between soil (A-horizon) DOM composition and bacterial metabolic responses around decomposing pig carcasses.

PLS-R biplot (**A**) of chemical (explanatory) versus bacterial (response) variables measured in the soils sampled at varying lateral distances. Linear regression (**B**) of PLS-R t1 and u1 scores categorized by sampling distance and season. Score plots for variables of importance to projection (VIP) that contributed to the t1 (**C**) and t2 (**D**) components of the PLS-R model.

Discussion

Spatial patterns

Large mammalian carrion comprise approximately 20% of carbon in mass and have been estimated to contribute over 1% of organic matter inputs in some terrestrial environments (Carter et al., 2007). It was therefore unsurprising that we observed a localized increase in soil DOC during the summer season, following the decomposition of surface deposited pig carcasses (day 55). Levels were greatest beneath the carcasses (0 cm), followed by a sampling distance of 20 cm uphill. Soil DOC enrichment has likewise been recorded in soils sampled directly underneath animal and human remains (Chowdhury et al., 2019; Fancher et al., 2017; Keenan et al., 2018; Macdonald et al., 2014; Woelber-Kastner et al., 2021). Though, small increases in DOC were dissimilarly recorded up to 5 m away from pig carcasses placed on principally clayey soils in Texas (Heo et al., 2021). The diffusion of decomposition products was furthermore anticipated to follow the slope of our experimental terrain since Aitkenhead-Peterson *et al*. (2012) reported a lengthy downhill leaching of DOC from cadavers. We therefore did not foresee an uphill increase. The slope of our terrain may not have been sufficient to influence the directionality of leaching. Instead, the location of carcass rupture, the displacement of the carcass during bloating and the movement of maggot masses may have had a greater effect. We moreover found that DOC increases were vertically bound to the A-horizon (≤14 cm). A limited diffusion of cadaver DOC down the soil profile was also reported by Fancher *et al*. (2017), who observed concentration changes only up to depths of 15 cm. Interstudy comparisons between biogeographic regions must however be done with caution since differences in soil type, precipitation, cadaver mass, adipose deposits, ante- or peri-mortem trauma, scavenging and bacterial mineralization can all influence the spatial extent of CDI measures (Aitkenhead-Peterson et al., 2012; Barton et al., 2020; Coe, 1978; Damann et al., 2012; Heo et al., 2021). Within the context of our study, the elevated DOC levels were particularly interpreted as a spatially constrained increase in soil DOM quantity.

Optical analyses revealed that the spatial pattern in DOM quantity was accompanied by an increase in quality. This was seen by a rise in the relative DOM fluorescent signal for labile amino acids (tyrosine, tryptophan). Similar protein-like fluorescence has been observed in an Alaskan creek following annual salmon spawning mortalities (Hood et al., 2007). Such

compositional changes could be partially attributed to the degradation of protein-rich soft-tissue, as shown by Nolan *et al*. (2020) who identified known blood and tissue components in the peptide profile of porcine decomposition fluid. However, the turn-over rate of free amino acids in soils is universally rapid due to nitrogen limitations (Macdonald et al., 2014). By the time the first summer soil core was collected, a portion of carcass-derived proteinaceous DOM may have already been assimilated into microbial biomass or lost to mineralization. Keenan *et al*. (2019) reported CDI ^{15}N isotopic profiles that are suggestive of both carrion and microbial inputs, and Macdonald *et al*. (2014) attributed only 40% of N-inputs to carrion-derived free amino acids and peptides. The positive correlation we observed between protein-like fluorescence (C3) and the freshness index (BIX) (similarly indicated that the proteinaceous DOM in our CDI soil was mostly microbial in origin. These protein inputs may have also been responsible for the depletion in phenol-like DOM compounds. Phenolic compounds, like tannins, are naturally introduced to soil systems by plant root exudates or decaying leaf litter (Schmidt et al., 2013). Proteins, such as those generated during CDI formation, can polymerize with these endogenous phenols to produce recalcitrant humic acids. This phenomenon has been especially observed during composting and is known as the phenol-protein theory of humification (Sánchez–Monedero et al., 1999; Wu et al., 2017). The conversion of phenols to humic substances would moreover explain the negative correlation that we obtained between phenol-like (C4) and humic-like (C2) DOM fluorescence. Altogether, fluorescence spectroscopy was capable of detecting distinct changes in DOM chemical quality within CDI soils and further highlighted the important implication of microbial sources in the development of labile and humic DOM fractions following body decomposition.

Topsoil within 20 cm laterally from the carcasses also exhibited elevated bacterial activity (respiration) and cell abundance. Cell counts could have been affected by stimulated cell growth from labile inputs or from the introduction of carcass-associated gut bacteria, which have been found to persist in soil up to 198 days post-mortem (Cobaugh et al., 2015; Prescott & Vesterdal, 2021). Spatial patterns in BA nevertheless did not mirror the measured spike in BR, particularly at 0 cm, thus indicating a possible increase in cell-specific respiration. Resource dense environments, like CDIs, tend to select for copiotroph bacteria with high metabolic demands

(Strickland & Wickings, 2015). The selection for copiotrophic groups has been repeatedly observed in CDI communities, where Proteobacteria increasingly dominate following the purging of decomposition fluids. The surge in respiration could have similarly been produced by shifts in the metabolic strategies of native soil bacteria (Cobaugh et al., 2015; Finley et al., 2016; Metcalf et al., 2013; B. Singh et al., 2018). These marked increases in activity could have promoted rates of carbon mineralization and loss that were greater than the rate of CDI diffusion. This would explain the small spatial extent of carcass DOC. Other soil microbial communities, like fungi and protists, undoubtedly also contributed to CDI respiration. However, in this study we decided to isolate and focus on the response of prokaryotic groups that dominate CDI soils and labile DOM decomposition (Chapin et al., 2002; Strickland & Wickings, 2015). Non-bacterial groups should be included in future studies to reveal competitive and trophic interactions that can potentially influence CDI outcomes.

Biolog EcoPlate™ assays also demonstrated a shift in bacterial metabolic potential. Some soil bacteria in proximity to the carcasses preferentially utilized carbohydrate substrates. A similar response has been seen in soils amended with other high-quality resources like animal manure and biochar (Ayaz et al., 2022; Chakraborty et al., 2011). Microbial community structure and function are highly related (Fuhrman, 2009), therefore the reduction of labile carbohydrates could echo the high energy needs of a growing copiotroph population. Glucose-1-Phosphate (C8) and D-Cellobiose (C7) were carbohydrates that were notably degraded by CDI communities. The former was previously identified as a likely metabolic indicator of pig carcass decomposition by Heo *et al*. (2021), whereas the latter may reflect the metabolism of pig-derived cellulolytic bacteria (Checcucci et al., 2021). The metabolic preference for these substrates may therefore be functional markers of a bacterial community that is associated with pig carcass decomposition.

Despite the characterized increase in labile protein-like DOM, the CDI bacterial community did not demonstrate a preferential utilization of EcoPlate™ amino-acid substrates. Fluorescence spectroscopy is unfortunately restricted in the detection of carbohydrate compounds, so we were unable to assess their contribution to the labile DOM pool. Bacteria can however have a variable preference for carbohydrates and amino acids, regardless of the relative substrate quantities. The differential use of carbohydrates and proteins can arise from fluctuations in resource C:N,

substrate allocation (energy VS biosynthesis), the availability of non-protein nitrogenous compounds (e.g., ammonia), substrate accessibility, and/or physiological adaptations (Chapin et al., 2002; Weiss & Simon, 1999). This isn't to say that a metabolic preference for amino acids did not occur earlier in CDI formation, prior to summer sampling.

Differential DOM composition from leaf-litter and root exudates has been well-established as a regulator of microbial patchiness in forest soils (de Graaff et al., 2010; Tian et al., 2015; Yano et al., 2005). Labile compounds from these sources are particularly recognized to stimulate bacterial metabolic activity through the alleviation of energy and nutrient limitations (Bray et al., 2012). We found that this DOM-bacterial relationship remained true in CDI soils. Partial least squares regression analysis listed high DOM quantity (DOC) and quality (%C3, BIX) as important predictors of bacterial activity (BR, BA) and function (metabolic potential). A strong gradient over sampling distance was also seen in the relationship between the model scores for DOM composition (t1) and bacterial responses (u1). This spatial connection between carcass input chemistry and bacterial trends reinforces the view of a biochemical “hot spot” and further suggests that DOM composition is worth considering when examining microbial variabilities in CDIs.

Temporal and seasonal patterns

The biochemical disturbances described above were found to be temporally short-lived. As hypothesized, heightened bacterial activity and functional changes led to the rapid consumption and transformation of carcass inputs. We saw a steep decrease in soil DOC levels between summer and autumn (day 156), but concentrations still remained slightly more elevated than non-CDI soils (>20 cm). This decline was surely because of the atmospheric loss of CO_2 and other carbon-containing gases evolved from bacterial respiration (Putman, 1978). The decrease in quantity also coincided with a secondary shift in DOM quality, where the composition became increasingly characterized by microbial humic-like compounds instead of labile proteins. As labile DOM inputs were being respired for energy use, some of it would also have been microbially converted into bulky biomass components, metabolites, polymeric slimes, or polymerizing intermediates (Guggenberger, 2005; Strawn et al., 2015). These residues could have then

contributed to the growing fraction of microbial humic DOM. The accumulation of recalcitrant microbial products has been reported following labile additions from plant matter (Cotrufo et al., 2013; Tamura & Suseela, 2021; Tamura & Tharayil, 2014). But to our knowledge, this is the first time that a temporal switch from labile to humic DOM has been observed as the result of inputs and microbial activity associated with terrestrial animal decomposition. This supports the theories of Strickland and Wickings (2015) who suggested that the microbially mediated stabilization of organic matter is likely to occur following labile carcass inputs. These evolved CDI humic substances could have implications in generating patches of improved soil structure, aeration and water holding capacity by promoting the formation of stable aggregates (Chaney & Swift, 1984; Foth, 1990).

Ambient temperature can be an important temporal regulator of soil microbial processes, especially in temperate regions that experience extreme seasonal variabilities (Birgander et al., 2018; Kritzberg & Bååth, 2022). Freezing temperatures can limit the availability of liquid water and decrease enzyme reaction rates (Arcus & Mulholland, 2020; Drotz et al., 2009). This is why microbial processes are often assumed to slow-down or cease in sub-zero conditions. Even with these temperature-related pressures, we found that CDI bacterial responses were maintained throughout the cooler autumn season. Insulation from fat deposits and snow coverage likely aided in decoupling ambient and soil temperatures by helping to retain heat. Organic matter inputs also likely contributed to the freezing-point depression of water by lowering matric and osmotic potentials (Drotz et al., 2009). Together, this could have buffered CDI microbial communities from freezing above-ground conditions. This would explain why BR and BA, although reduced from their summer values, remained noticeably elevated when compared to the surrounding soil. Spikes in CO_2 efflux and bacterial cell counts have similarly been seen in soils under mummified seal remains in an annually cold Antarctic environment (Tiao et al., 2012). Our results further suggest that the insulative effect of decomposed carrion also extends to the metabolic profile of soil bacteria. CDI communities managed to maintain their metabolic preference for carbohydrates, even when faced with an almost 20°C difference in ambient temperature between the summer and autumn seasons. This metabolic consistency differs from Pechal *et al*. (2013), who attributed temperature variabilities to their observed differences in

seasonal and annual Biolog profiles of pig carcasses. This suspected temperature sensitivity was however seen in samples taken from the skin and buccal cavity, which are areas that are more directly exposed to environmental conditions. Rather, the results of our study suggest that decomposed carrion may provide a protective effect to soil bacteria and help to reduce temperature-dependent shifts in soil bacterial metabolic activity.

The persistence of bacterial activity through the cooler months was also evident in the DOM composition of spring samples (day 324). By this point, DOC concentrations in CDI soils had returned to almost baseline levels, though DOM remained distinctly characterized by microbial humic compounds. This could imply that losses in DOM quantity were related to microbial processes that were sustained throughout the autumn and winter. Snowmelt could have had a dilution effect on DOC, and the presence of anti-scavenging cages may have also obstructed leaf coverage that could have replenished vegetal humic- and phenol-like DOM. Nevertheless, evidence of continued bacterial respiration, growth, and enzyme synthesis under sub-zero conditions has been repeatedly shown in soil incubation studies and arctic field experiments (Drotz, Sparrman, Nilsson, et al., 2010; Drotz, Sparrman, Schleucher, et al., 2010; McMahon et al., 2009; Michaelson & Ping, 2003; Nikrad et al., 2016). It is therefore not unreasonable to assume that soil bacteria continued to transform carcass inputs under Quebec (Canada) winter conditions. Persistent and even elevated bacterial activity throughout the autumn and winter seasons are likely to become more pronounced as global temperatures continue to rise in the face of climate change. This is expected to generate accelerated rates of carcass decomposition and soil DOM processing, thus altering the above reported temporal CDI trends (O'Donnell et al., 2016; Parmenter & MacMahon, 2009).

The resulting spring depletion in DOM quantity and quality meant that bacterial activity was no longer optimally supported. CDI respiration rates were further reduced towards or below control values, even in the presence of inflated cell counts. This could have emerged from a decrease in cell specific respiration, but it must be noted that we did not stain for viability. Therefore, BA likely constituted both living, dead and dormant cells. Spring CDI microbial communities additionally displayed a decrease in substrate class specificity and began to target a broader range of less energetically favourable compounds (e.g., carboxylic acids, phenolic

compounds). CDI substrate utilization patterns were less distinct and resembled more the profiles of non-affected soils. The exhaustion of labile DOM inputs may have facilitated the reestablishment of some endogenous phyla consisting of metabolically diverse oligotrophs (e.g., Acidobacteria). But it was unlikely that CDI soils fully returned to their native community. Alterations in bacterial community composition have been detected in soils as far as 10 years after swine carcass decomposition (Burcham et al., 2021). The spring convergence in Biolog profiles between CDI and undisturbed soils could have resulted from functional redundancies that emerged from shifting metabolic pathways. This idea is supported by Singh *et al*. (2018) who saw a recovery in mineralizable carbon and citric acid respiration despite a prolonged (732 days) disruption in CDI community composition. Whether our observed metabolic profiles and activity were because of community assembly or individual strategies, they demonstrated that CDI bacteria were functionally restored almost a year after carcass placement.

Conclusion

Our study demonstrated a distinct, long-lasting, and spatially constrained disturbance to soil DOM chemical composition following the decomposition of pig carcasses in a Canadian forested environment. The high quantity and quality of DOM that was initially inputted into the soil was either progressively respired as CO_2 or transformed to recalcitrant humic-like compounds by bacterial communities. Throughout the entirety of the study, DOM characteristics remained discernible from the vegetal humic signal of unaffected soils. Soil DOM composition may therefore be a suitable chemical marker for detecting and mapping areas affected by body decomposition, even in later stages of decay.

Soil DOM composition was moreover found to be an important driver of bacterial responses to carcass decomposition. Large amounts of labile compounds first favored elevated rates of bacterial respiration and carbohydrate metabolism. These bacterial changes persisted over the cooler autumn and winter months, which also contributed to the sustained consumption and transformation of DOM inputs. The eventual depletion of labile DOM was then seen to be concurrent with the functional recovery of soil bacterial communities by the spring season.

These findings deepen our understanding of the relationship between animal decomposition, soil chemistry and microbial ecological processes, particularly the cycling and stabilization of organic matter within a cold-climate ecosystem. The study of individual carcasses and the exclusion of scavengers provides a snapshot of the maximum effect a CDI can produce in a temperate forested environment. This can serve as a basis to hypothesize outcomes in scenarios where tissue loss and dispersion occurs from scavenging or larger spatial inputs are generated from mass mortalities. Our findings add to a growing conceptual framework of carrion's ecological contribution. Such information will be crucial in managing ecosystem health following fluxes in the distribution and density of animal populations produced by anthropogenic and climatic factors (e.g., livestock rearing, wildfires, mass starvation) (Barton et al., 2019).

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Supplementary material

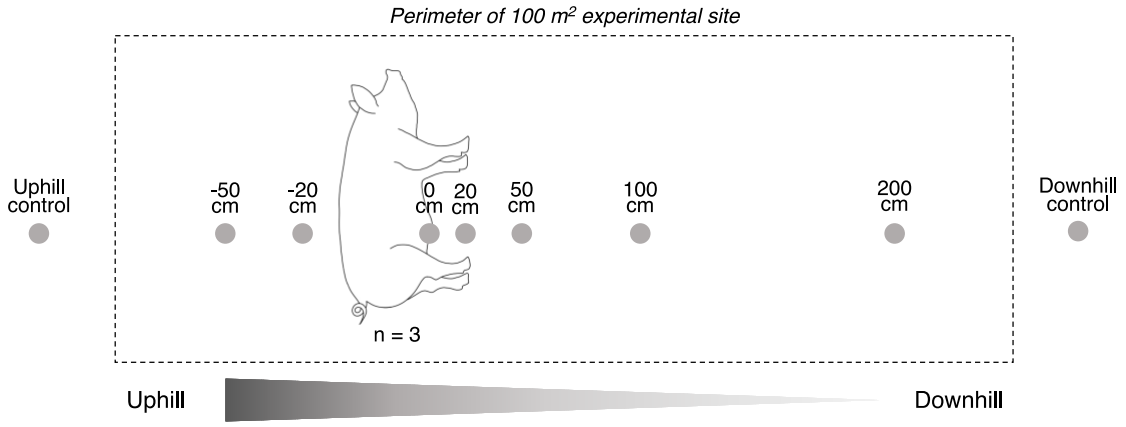

Figure S2.1 – Soil sampling design for UQTR pig study.

Soil cores were taken at incremental distances from decomposing pig carcasses (*n*=3). Soil was sampled at distances of 0, 20, 50, 100 and 200 cm laterally downhill from the carcasses. Two samples were also taken 20 and 50 cm laterally uphill from the carcasses, and are denoted as negative distances (-50, -20 cm). Uphill and downhill control samples were collected outside the fenced perimeter of the 100 m^2 experimental site.

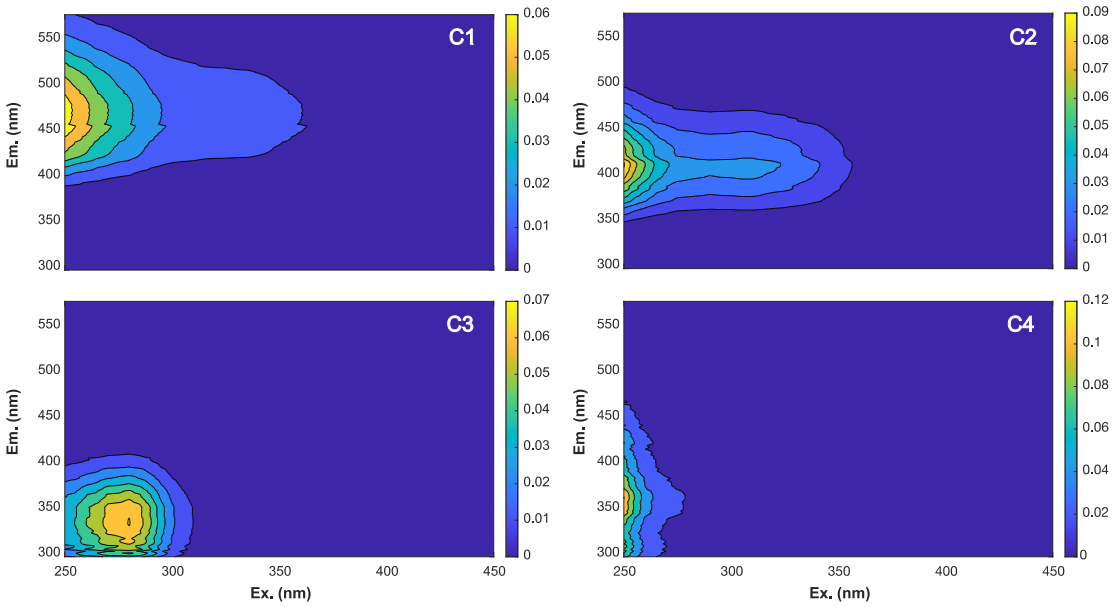

Figure S2.2 – DOM fluorescent fingerprint of validated PARAFAC components (C1-C4) generated from soils sampled around decomposing pig carcasses.

Components were identified as terrestrial humic-like (C1), microbial humic/fulvic-like (C2), protein-like (C3) and phenol-like (C4).

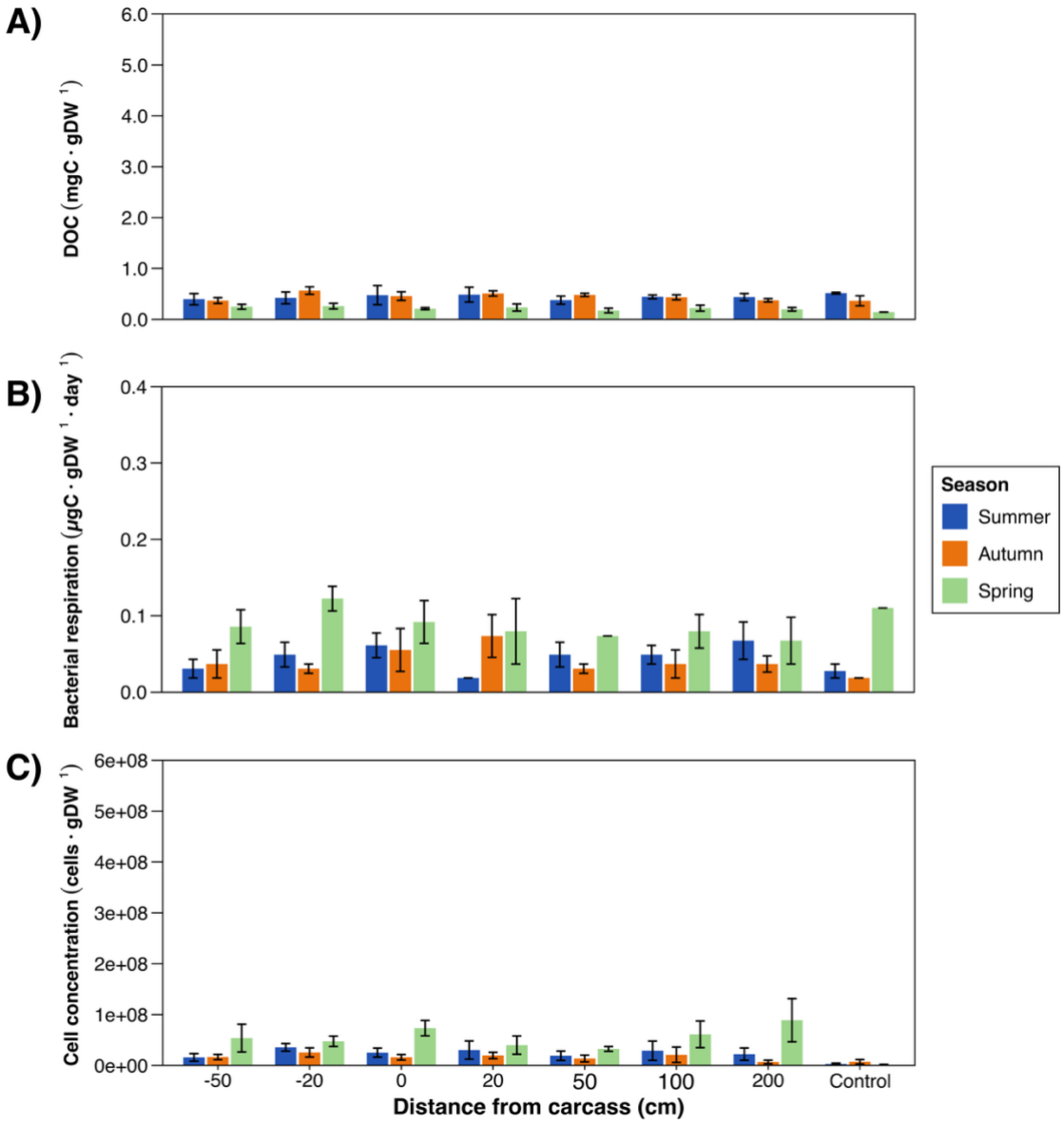

Figure S2.3 – Seasonal trends in soil (B-horizon) DOC and bacterial activity around decomposing pig carcasses.

Mean and standard error of DOC (**A**) bacterial carbon respiration (**B**) and bacterial cell abundance (**C**) from the B-horizon of soils sampled at varying distances from decomposing pig carcasses (*n* = 3). Scaling reflects figures 2.1 and 2.3 to facilitate comparison between soil horizons. Control samples taken outside of the experimental site are included as the furthest distance from the carcass.

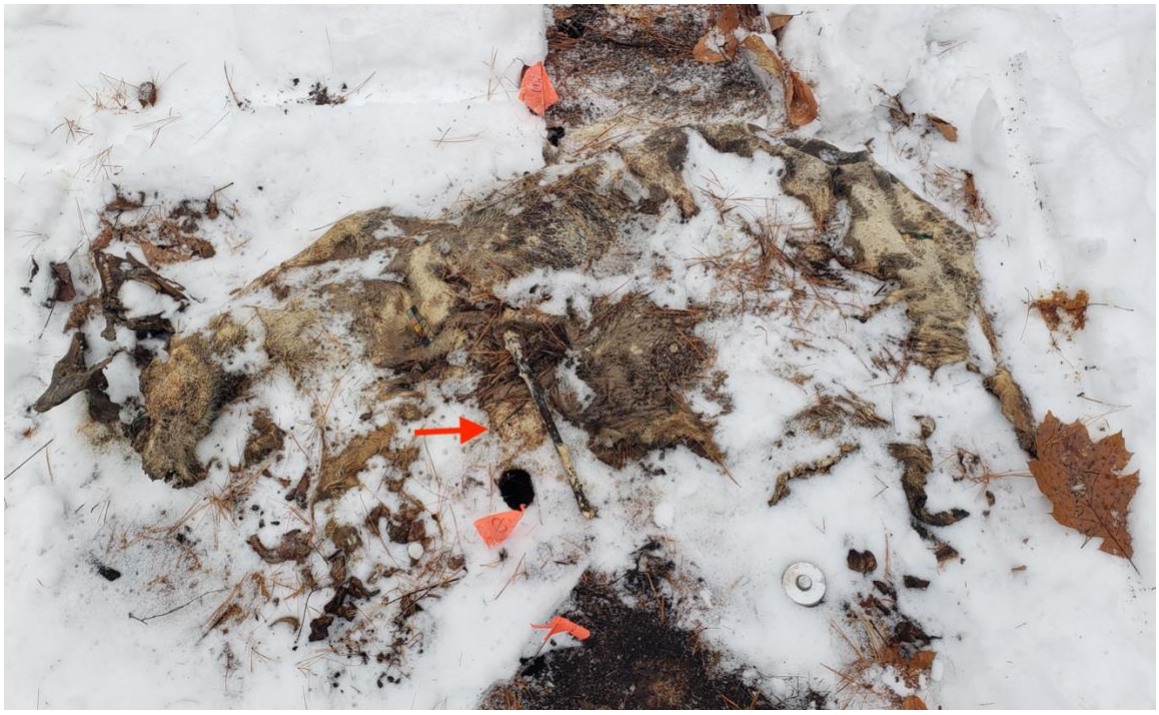

Figure S2.3 – Visual appearance of a pig carcass during autumn sampling (day 156).

Note the snow coverage and presence of fat deposits (red arrow) which may have had a protective effect on soil temperature and bacterial activity/function.

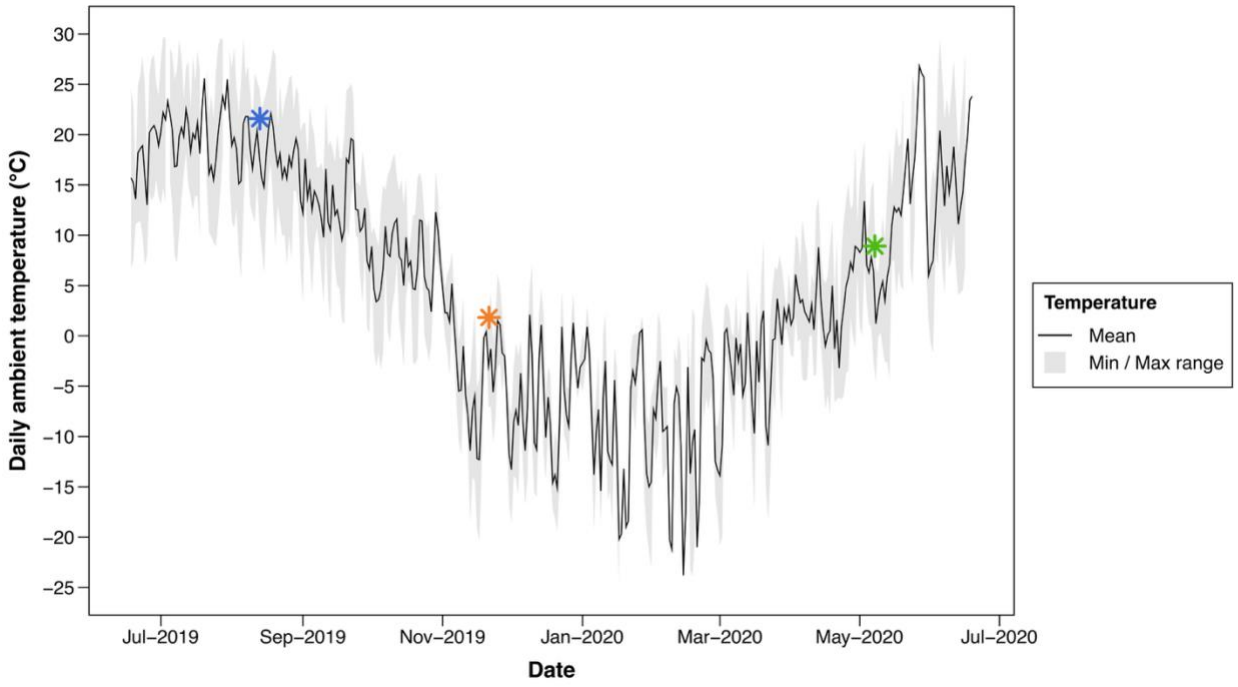

Figure S2.5 – Ambient temperature in Trois-Rivières, Quebec between 2019-2020.

Mean daily ambient temperature (solid line) recorded from a nearby weather station (WMO ID: 71729) over a one-year period following pig carcass placement (18 June 2019). Shaded area represents the minimum and maximum daily temperature ranges. Soil sampling dates for the summer (12 August 2019, Day 55), autumn (20 November 2019, Day 156) and spring (6 May 2020, Day 324) are respectively indicated by a blue, orange, and green asterisk (✳).

Table S2.1 – Biolog EcoPlate™ substrate codification.

Adapted from Checcucci *et al*. (2021) and Sala *et al*. (2010).

Identification code	Chemical class	Substrate
A1	Amine	Phenylethylamine
A2	Amine	Putrescine
AA1	Amino acid	L-Arginine
AA2	Amino acid	L-Asparagine
AA3	Amino acid	L-Phenylalanine
AA4	Amino acid	L-Serine
AA5	Amino acid	L-Threonine
AA6	Amino acid	β -Hydroxy-Glycyl-L-Glutaminic Acid
C1	Carbohydrate	β-Methyl-D-Glucoside
C2	Carbohydrate	D-Galactonic Acid γ-Lactone
C3	Carbohydrate	D-Xylose
C4	Carbohydrate	i-Erythritol
C5	Carbohydrate	D-Mannitol
C6	Carbohydrate	N-Acetyl-D-Glucosamine
C7	Carbohydrate	D-Cellobiose
C8	Carbohydrate	Glucose-1-Phosphate
C9	Carbohydrate	α-D-Lactose
C10	Carbohydrate	D, L-α-Glycerol Phosphate
CA1	Carboxylic acid	Pyruvic Acid Methyl Ester
CA2	Carboxylic acid	D-Galacturonic Acid
CA3	Carboxylic acid	γ-Amino Butyric Acid
CA4	Carboxylic acid	D-Glucosaminic Acid
CA5	Carboxylic acid	Itaconic Acid
CA6	Carboxylic acid	α-Keto Butyric Acid
CA7	Carboxylic acid	D-Malic Acid
P1	Polymer	Tween 40
P2	Polymer	Tween 80
P3	Polymer	α-Cyclodextrin
P4	Polymer	Glycogen
PC1	Phenolic compound	2-Hydroxy Benzoic Acid
PC2	Phenolic compound	4-Hydroxy Benzoic Acid

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Chapter 3 – Season matters: Timing of cadaver deposition influences soil biogeochemical changes in a temperate human taphonomic facility.

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Abstract

Human taphonomic facilities (HTF) are outdoor spaces dedicated to research, educational and training activities related to human decomposition. Through the purging of decomposition by-products, a body within a HTF will generate a chemically and microbially disrupted zone of soil known as a Cadaver Decomposition Island (CDI). The biochemical dynamics of CDIs have been minimally investigated across contrasting seasons, thereby impeding our understanding of how cadaveric inputs are processed within temperate soils. This gap was addressed in a Canadian HTF by examining soil organic matter (SOM) chemistry and bacterial metabolic responses within the CDIs of donors bodies who were deposited under warm or cold seasonal conditions. Decomposition generated a pulse of SOM that was first rich in carbon then nitrogen. This was associated with an enrichment in $\delta^{13}C$ and $\delta^{15}N$ that was attributable to cadaveric tissues and enhanced mineralization. Fluorescence spectroscopy also revealed a transition in the molecular characteristics of organic matter that was indicative of a shift from recalcitrant terrestrial substances to labile microbial compounds. In addition, bacteria were observed to preferentially utilize carbohydrate substrates for energy production. These effects arose during the Active–Dry phases of decomposition, occurring between 13–139 days for warm deposited donors, but were delayed (31–364 days) and reduced in magnitude for cold donors. All changes were limited to the A-horizon and within a sampling radius of 20 cm from the body. These findings will inform temperate HTFs of their potential environmental impact and direct the development of soil-based forensic techniques.

Keywords : Forensic taphonomy, Human taphonomic facilities, Cadaver decomposition island, Soil organic matter, Soil biogeochemistry, Nutrient cycling, Bacterial responses.

Highlights

- Effect of human decomposition on soil biochemistry was spatially constrained.
- Increases in soil carbon preceded nitrogen.
- Bacterial processing altered soil organic matter lability.
- Cadaveric inputs enhanced bacterial mineralization but supressed growth.
- Cold season deposited led to delayed and reduced soil changes, even after warming.

Introduction

Human taphonomic facilities (HTF) are outdoor installations where semi-controlled research, educational and training activities pertaining to human decomposition can safely and ethically take place (Pecsi et al., 2020). HTFs are primarily centered around the development of regionally specific techniques for medicolegal death investigations, and the recovery of victim remains. The majority of HTFs are located within the continental United States, with additional sites found in Australia, Canada and the Netherlands (S. Forbes, 2017; Pecsi et al., 2020; Wescott, 2018). The recent growing interest in forensic taphonomic research (Varlet et al., 2020) paired with the increasing popularity of whole-body donation (Bagian et al., 2024; Bolt et al., 2010), means that most HTFs have a continuous presence of donor remains on their land. With each deposited donor, a cadaver decomposition island (CDI) is established in the surrounding soil. A CDI is typically formed following the purging of moisture, organic compounds and bacteria from the body (Carter et al., 2007; Cobaugh et al., 2015). Additional pulses of organic matter are introduced into the soil from the excretions, secretions and metabolites of colonizing insects and microbial decomposers (Strickland & Wickings, 2015). CDIs are considered a natural disturbance since the leaching of these materials can generate localized changes in soil chemistry (e.g. nutrients, carbon, pH) and microbiology (e.g. activity, function, community) (Carter et al., 2007; Cobaugh et al., 2015). These biochemical changes are garnering interest for their potential use as forensically relevant markers for postmortem interval estimation, clandestine grave detection and for determining body displacement (Fiedler et al., 2023; Wescott, 2018). There is also great apprehension towards the approbation of new facilities, as government regulatory bodies question the role CDIs play in an HTF's ability to comply with regional environmental standards (Pecsi et al., 2020; Varlet et al., 2020). Examining CDIs within HTFs from an ecological perspective can help address both environmental concerns and the need for advancing soil-based forensic techniques.

Human remains are known to offload a complex mixture of organic residues (S. L. Forbes & Carter, 2015). Upon leaching, these residues contribute to soil organic matter (SOM), which serves as a source and reserve of energy/nutrients in terrestrial systems. The microbial cycling of

SOM across trophic levels is crucial for supporting ecological processes like primary productivity (Wardle et al., 2004). The ease and rate at which SOM is processed (i.e. lability) is dependent on several factors including its chemical characteristics (e.g. aromaticity, conjugation, molecular weight, stoichiometry) and the metabolic state (activity, function) of microbial communities (Chapin et al., 2002; D'Andrilli et al., 2019). Dissolved organic matter (DOM) is the soluble and most biologically available fraction of SOM (Chenu et al., 2014). Bacteria are particularly adept at responding to shifts in DOM inputs and are most involved in degrading its labile constituents (D'Andrilli et al., 2019; Goldfarb et al., 2011). Although DOM represents a small percentage of the total SOM pool, its biological reactivity accounts for an important portion of carbon and nitrogen mobilization (Gmach et al., 2020; Li et al., 2021). It also serves as a vital chemical modulator of soil structure and the retention of nutrients, heavy metals and xenobiotics (Gmach et al., 2020; James & Harrison, 2023). The chemical structure and associated lability of DOM is highly influenced by its source and biochemical transformation. Disruptions in DOM chemistry and its microbial processing can lead to ecosystem decline from the downstream imbalance in energy and nutrients budgets. Therefore, ecologists often monitor DOM and bacterial responses as an indicator of environmental perturbation and health (Huang et al., 2021; Stefanowicz, 2006). A similar approach can therefore be adopted within HTFs to track CDI inputs and how they are being microbially utilized and redistributed throughout the ecosystem. This can provide a more comprehensive understanding of the bulk energy/nutrient disturbances cause by cadaver decomposition and the potential consequences on HTF environmental integrity.

Decomposition is highly influenced by several abiotic factors. The importance of ambient temperature on body decomposition has been well established and is recognized for its effect on decomposition-associated microbial and insect activity (Iancu et al., 2024). Moisture and the availability of liquid water is commonly cited as the second most influential taphonomic factor due to its role in regulating microbial metabolism by affecting the diffusion of oxygen and substrates (Carter et al., 2010; Mann et al., 1990). Similar to body decomposition, soil moisture and temperature affect the microbially-mediated degradation and cycling of SOM (Kumar & Karthika, 2020). Both of these factors, and others, can vary considerably over season especially in northern temperate regions that experience drastically different summer and winter climatic

conditions. The majority of published work has however only been conducted under warm conditions due to the lower latitude position of most HTFs and the out-dated assumption that decomposition processes cease at below freezing temperatures (Cockle & Bell, 2017; Megyesi et al., 2005; Ribéreau-Gayon et al., 2023). Information is therefore lacking on how taphonomy differs during cooler seasons and how that may translate to variation in CDI biogeochemistry and microbial processing (Meyer et al., 2013).

In the following study, we aimed to investigate the impact of CDIs under contrasting seasonal conditions within a Canadian temperate HTF. This was achieved by spatiotemporally examining SOM (DOC, DOM fluorescence spectroscopy, $\delta^{15}N$, $\delta^{13}C$, C/N) and bacterial (abundance, respiration, biomass production, growth efficiency, metabolic potential) dynamics in the CDI of donors deposited during warm or cold seasonal conditions. Compared to vegetation, animal and microbial residues are recognized as high-quality and labile resources due to their N-rich peptides, higher lipid content and lack of complex structural compounds (e.g. lignin, hemicellulose) (Bagian et al., 2024; Chapin et al., 2002; Cobaugh et al., 2015). We therefore hypothesized that body decomposition would supply large quantities of labile DOM, which would be used to fuel soil bacterial activity and growth. The onset and recovery of these changes were further expected to be delayed for donors deposited in cooler conditions due to the slowing of gross body decomposition and microbial metabolism. Findings from this study will provide insight into how seasonal variations affect the processing of cadaveric inputs and their resulting biochemical disturbance within CDI soils. This will be particularly valuable for ensuring that new temperate HTFs conform to environmental regulations and adopt appropriate mitigation strategies if needed. Results may also reveal forensically relevant biomarkers of decomposition and help to develop a more mechanistic understanding of their persistence and distribution.

Materials and methods

Experimental set-up and sampling

The following study took place at the site for Research in Experimental and Social Thanatology / *Recherche en sciences thanatologiques, expérimentales et sociales* (REST[ES]); a HTF that is associated with the Université du Québec à Trois-Rivières (UQTR). The 1600 m^2 facility

is situated in the Industrial Parc and Port of Bécancour within the Saint-Lawrence Lowlands ecoregion of Québec, Canada. The area experiences long cold winters and short warm summers (**Figure S3.1**), which corresponds to a *Dfb* climate by the Köppen-Geiger system (Peel et al., 2007). REST[ES] lies within a young (30-50 years) mixed temperate woodland dominated by maple trees (*Acer spp*.), birch trees (*Betula spp*.), white spruce (*Picea glauca*), perennial herbaceous plants (e.g. *Maianthemum canadense*) and ferns (e.g. *Dryopterus carthusiana*). The terrain is minimally sloped (≤ 3%) and punctuated by shallow depressions which become temporarily inundated following the spring snow melt. The soil is an acidic gleyed humo-ferric podzol with a sandy-loamy A-horizon (0-13 cm) and loamy-sandy B-horizon (13-38 cm) (Choinière & Laplante, 1948; Soil Landscapes of Canada Working Group, 2010).

Six adult donors were accepted between August 2020 and May 2021 through the willed body donation program of UQTR's Anatomy Teaching and Research Laboratory (**Table 3.1**). Donors were only accepted if they presented with no communicable diseases, embalming, major bodily trauma, emaciation or visible signs of decomposition. All bodies were kept refrigerated (4°C) prior to being surface deposited, undressed, in a supine position. Galvanized mesh anti-scavenger cages were placed over the donors when sampling was not taking place. Ambient temperature data was recorded from an onsite HOBO U30 weather station (Onset Computer Corporation, Bourne, MA). This data was used to calculate, according to the methods of Megyesi et al. (2005), the accumulated degree days (ADD) for each sampling event. Donors were either categorized as being deposited during a warm (summer, spring) or cold (autumn) seasonal period. The cold period was distinguished from the warm if donors experienced below freezing (< 0°C) temperatures and required over 3000 ADDs to reach the final phase of decomposition (dry remains).

Soil was collected from around each donor using a handheld soil probe (2.5 X 30 cm). Sampling was done at incremental distances uphill (+50, +20 cm) and downhill (20, 50, 100 cm) from each body to capture the lateral dispersion of the CDI. Soil was also collected directly beneath (0 cm) the body by carefully rolling the donor onto their side (**Figure S3.2**). Soil cores were wrapped in aluminum foil and immediately transported to UQTR for refrigeration at 4°C.

Sampling was repeated during each visible stage of decomposition: Fresh, Active decay, Advanced decay and Dry remains. Donor D05 did not undergo Active decay due to cold temperatures and Active samples for donor D01 were lost due to contamination during the drying process.

Soil cores were processed by discarding the organic layer and dividing the A-horizon and B-horizon into separate aluminum dishes. The A-horizon was prepared for all sampled distances, whereas only soil from +20, 0 and 20 cm were prepared for the B-horizon. This choice was made since the vertical leaching of decomposition products was likely to occur only in close proximity to the body, as observed in a prior pilot study (Pecsi et al., 2024). Soil was dried for one week at room temperature (~ 20°C) under a fume hood. Dried soil was then sieved (2 mm) to remove debris and aggregates prior to being refrigerated (4°C) in sealed plastic bags.

A 1:40 soil solution in ultrapure water was shaken overnight at 4°C. Extracts were left upright for a minimum of 30 minutes to allow soil particles to settle. The supernatant was removed and mixed to a final concentration of 0.001N $NaHCO_3$ to lightly buffer the sample and replicate the ionic strength of natural soil systems (Wickland et al., 2007). Buffered extracts were then passed through pre-combusted (500ºC, 4 hrs) GF/F (0.7 µm) or GF/D (2.7 µm) glass microfiber filters, respectively, for chemical and bacterial analyses. Remaining air-dried soil was further oven-dried at 105°C for 24 hrs for isotopic/elemental analyses and to correct data for residual moisture content.

Chemical analyses

Dissolved organic carbon (DOC) was measured in acidified (HCl, pH < 2) GF/F filtrates using a Sievers M9 Portable TIC/TOC Analyzer (GE Analytical Instruments, Boulder, CO). Instrument verification was done against a five-point potassium hydrogen phthalate calibration curve and sample readings were taken in triplicates with an accepted relative standard deviation of ≤ 10%.

A subset of oven-dried soil was ground to a fine powder using benchtop ball mill (Mixer Mill MM 30, Retsch, Haan, Germany). 25 mg of ground soil was encapsulated in tin combustion cups for elemental (C, N) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses using a PYRO cube® (Elementar,

Langenselbold, Germany) elemental analyzer interfaced to a Delta V Plus (Thermo Scientific, Waltham, MA) isotope ratio mass spectrometer. Reference materials USGS 40 and USGS 41 were used for calibration and the normalization of isotopic values to the working standards of air-N_2 and Vienna Pee Dee Belemnite scales. Analytical precision for C and N was ± 0.3‰.

The optical properties of DOM are a product of its chemical composition and reflects its lability, source and degree of biological, chemical and physical degradation (Hood et al., 2007; Stedmon & Markager, 2005). Absorbance and fluorescence measures were therefore used to infer information on DOM chemical characteristics and biochemical processing. Fluorescent excitation-emission spectra (EEM) of GF/F filtrates were generated using a 1 cm glass cuvette and a Carey Eclipse Fluorescence Spectrometer (Agilent, Santa Clara, CA). EEMs were collected between excitation (Ex) wavelengths of 230-540 nm and emission (Em) wavelengths of 300-600 nm, respectively, at 5 nm and 2 nm intervals. Instrument bias was corrected using manufacturer provided files, whereas the inner filter effect was corrected using the absorbance spectra (200-800 nm) of GF/F filtrates that were analyzed on a Carrey 100 UV-Vis Spectrophotometer (Agilent, Santa Clara, CA) with a 1 cm quartz cuvette. Ultrapure water was used for baseline corrections and to normalize spectra to Raman units (R.U., nm^{-1}) based on the area under the Raman scatter peak at Ex = 350 nm.

Fluorescence- and absorbance-based indices were calculated as described in Fellman et al. (2010) and Hansen et al. (2016). The biological index (BIX) was used as an indicator of recently produced labile microbial DOM (Huguet et al., 2009), whereas the humification index (HIX) designated the presence of aromatic and high molecular weight humic substances (Zsolnay et al., 1999). The fluorescence index (FI) specified the relative contribution of aromatic and non-aromatic compounds associated with terrestrial plant matter and microbial activity (Cory & McKnight, 2005; Fellman et al., 2010). Finally, the spectral slope ratio (S_R) inversely corresponded to DOM molecular weight (Helms et al., 2008).

Parallel Factor Analysis (PARAFAC) was also performed on 188 EEMs to statistically identify components that represented distinct fluorescent groups within the DOM mixture. This was done using the drEEM toolbox 0.6.3 for MATLAB R2024b (Mathworks, Natick, MA) (Murphy

et al., 2013). A four component (C1-C4) model (**Figure S3.3**) with 99.25% explained variability was validated using split-half analysis (**Figure S3.4**). C1 (Ex_{max} 280 nm, Em_{max} 522 nm) corresponded with abundant terrestrial humic-like substances that are commonly associated with soils (Kothawala et al., 2014). C2 (Ex_{max} 270/360 nm, Em_{max} 458nm) was also attributed to terrestrial humic-like compounds but matched more specifically with UVC humic fluorophores that are found in forested environments (Fellman et al., 2010). C3 (Ex_{max} 310 nm, Em_{max} 416 nm) represented lower molecular weight fulvic-acids originating from microbial activity (Coble, 1996; Osburn et al., 2015; Yamashita & Tanoue, 2003). Lastly, C4 (Ex_{max} 280 nm, Em_{max} 316 nm) matched with the fluorescent signal of microbial amino acids (Coble, 1996; Huguet et al., 2009). Components are reported as their relative contribution (%C1-%C4) to the maximum fluorescent intensity (F_{max}, R.U.).

Bacterial analyses

Absolute cell counts for bacterial abundance (BA) were obtained using flow cytometry. 1.5 mL of GF/D filtrates were fixed to a concentration of 0.5% glutaraldehyde before being stored at -80°C. Thawed samples were stained to 2.5X SYBR™ Safe DNA gel (50X in DMSO, Invitrogen, Waltham, MA) and incubated in the dark for 15 minutes at room temperature (~20°C). Samples were analysed using a CytoFLEX S (Beckman Coulter, Brea, CA) equipped with a solid-state laser. Daily instrument performance and flow rate were verified according to manufacturer instructions using CytoFLEX Daily QC fluorospheres (Beckman Coulter, Brea, CA). Stained bacteria were enumerated to an acquisition threshold of 10,000 using an excitation wavelength of 488 nm and the log-scale of green (FITC, 525/40 nm) versus red (PerCP, 690/50 nm) fluorescence. CytExpert v2.6 software was used to perform manual gating, which was based on the cell counting strategy of Hammes & Egli (2005).

A SDR SensorDish™ and 4 mL vials equipped with PS5t optical oxygen sensors were used to determine bacterial respiration (BR) rates in GF/D filtrates. Vials were incubated in the dark and at room temperature (~20°C). Readings were automatically registered every 60 min for up to 1.5 weeks or until O_2 concentrations had plateaued. The rate of bacterial O_2 consumption was

calculated from the slope of O_2 ($mg \cdot L^{-1}$) plotted over time. This was converted to the rate of carbon consumption using a respiratory quotient of 0.95 (Hilman et al., 2022).

Bacterial biomass production (BP) was determined using the microcentrifugation method for leucine incorporation as outlined in Bååth et al. (2001). Volumes were modified to include 1.5 mL of GF/D filtrates and 20 μL of L-[4,5-^{3}H(N)]-leucine. Samples were incubated (~20°C, 1 hr) in the dark and terminated by the addition of 100 μL of cold TCA 100%. Following the washing steps and hot NaOH treatment, 1.3 mL of ScintiVerse™ BD Cocktail (Fisher Scientific, Hampton, NH) was added so that radioactivity could be measured on a HIDEX 300 SL liquid scintillation counter (Hidex Oy, Turku, Finland). The rate of carbon assimilated into bacterial biomass per unit of incorporated leucine was estimated using a conversion factor of 3.1 kgC $mmol^{-1}$ (Kirchman, 1993). Bacterial growth efficiency (BGE) was used to describe the proportion of carbon allocated toward bacterial energy expenditure versus biomass production. This was calculated as BGE = (BP / (BP + BR)) * 100.

Bacterial community function was evaluated based on the metabolic potential to degrade 31 sole carbon substrates (**Table S3.1**) included within the Biolog EcoPlate™ system (Biolog Inc., Hayward, CA). The 96-well plates contained a blank (water) and triplicates of each substrate spiked with a tetrazolium dye. 120 μL of GF/D filtrates were added to each well and stored in the dark at room temperature (~20°C). Purple colouration developing from substrate reduction was measured via absorbance at 590 nm using a Biotek Synergy H1 microplate reader (Agilent, Santa Clara, CA). Readings were repeated twice a day for the first week then once daily for the second week or until the average well color development (AWCD) of the entire plate plateaued. The optical density across triplicate wells were averaged, blank corrected and normalized to the incubation time at which the AWCD reached 0.5 to account for differences in inoculum density (Garland, 1997). Resulting negative values were considered zero. Due to time and cost constraints, only the 0 cm and 100 cm samples from the A-horizon were tested since these distances were expected to respectively represent the points most and least affected by body decomposition.

Statistics

Statistical analyses and graph building were done in RStudio version 4.3.1 (R Core Team, 2023). A linear mixed effects model (LME) (*lme4* 1.1-36) was used to analyze differences in mean values while controlling for inter-donor variability (random effect). LMEs were generated on log-transformed values to help improve normality. DOC was first modelled as a function of distance and phase (fixed effects), followed by a Tukey pairwise comparison (*emmeans* 1.11.0). This was repeated separately for each deposition season and soil horizon to help distinguish distance-related changes. Based on the DOC outcomes, the CDI was defined for all subsequent measures by averaging the +20, 0 and 20 cm values within each decomposition phase. Values for the remaining A-horizon samples (+50, 50 and 100 cm) were then averaged across all phases into a single "Non-CDI" reference value (**Figure S3.2**). This was done to isolate the spatial extent of the CDI and to increase statistical power. Temporal changes between CDI and Non-CDI values for C:N, $\delta^{15}N$, $\delta^{13}C$, PARAFAC components %C1-%C4, BA, BR, BP and BGE were analyzed separately for each soil horizon and deposition season using an LME as a function of decomposition phase (fixed effect). A two-sided Dunnett test with Bonferroni adjustment (*multcomp* 1.4-28) was applied to distinguish phase-related differences between CDI and Non-CDI samples of the A-horizon, whereas a Tukey pairwise comparison was used for the B-horizon since there were no reference samples (Non-CDI) analyzed at this depth. A principal component analysis (PCA) (*FactoMineR* 2.11) on scaled and centered data was additionally generated on DOM optical properties and Biolog data to respectively identify temporal shifts in DOM chemical characteristics and bacterial metabolic potential. Pearson correlations (*FactoMineR* 2.11) were used to assess the relationships between PCA loadings and principal components. A significance value of 0.05 was applied in all statistical analyses.

Table 3.1 – Human donor information and sampling timeline.

Donor	Age	Height (cm)	Weight (kg)	Deposition date	Deposition season	Sampling day (ADD, °C days)			
						Fresh	Active	Advanced	Dry
D01	55	172	68	10-Aug-2020	Warm	1 (48.73)	*N/A*	21 (408.66)	50 (796.18)
D02	71	173	70	10-Aug-2020	Warm	1 (48.73)	13 (284.40)	21 (408.66)	50 (796.18)
D03	69	165	54	28-Sep-2020	Cold	1 (39.34)	38 (288.71)	262 (1470.59)	364 (3478.52)
D04	79	152	79.4	05-Oct-2020	Cold	1 (24.24)	31 (197.30)	255 (1379.13)	357 (3387.01)
D05	72	180	85.2	02-Nov-2020	Cold	3 (9.03)	*N/A*	227 (1190.91)	329 (3198.84)
D06	77	177	79.5	11-May-2021	Warm	1 (21.26)	17 (286.21)	37 (646.93)	139 (2654.85)

Results

Body decomposition

The visual progression of body decomposition differed between donors who were deposited during warm and cold seasonal periods. Donors placed during warmer months proceeded through each decomposition phase as described in Maisonhaute & Forbes (2023). Generally, donors rapidly developed blue/green skin discoloration, marbling and abdominal bloating. The Active phase (13-17 days, 284-286 ADD) was characterized by sloughing of the epidermis and large maggot masses around the face, groin, armpit and body-soil interface. This was also accompanied by the purging of liquified tissues, which contributed to the formation of a darkened CDI. The Advanced phase (21-37 days, 409-647 ADD) was marked by significant volume loss, sinking of the abdomen, partial bone exposure (face, extremities) and a reduction in maggot masses. The skin dried to a leathery consistency with a glossy finish, dark brown/orange discolouration and patches of white mold growth. Mass loss continued into the Dry phase (50-139 days, 796-2655 ADD) and skin became increasingly darkened and matte. By this final phase, white mold patches persisted, and some additional bone exposure occurred around the neck and clavicle. CDI soil during the Advanced phase was punctuated by liquid and semi-solid fat deposits which became increasingly solidified during the Dry phase.

Donors placed during the colder months presented less drastic visual changes between the Fresh and Active phases (13-38 days, 197-289 ADD) of decomposition. Some darkening of the extremities, drying of the skin, green/purple marbling and sloughing of epidermis had developed, however maggot masses were minimal, little to no bloating occurred and a visible CDI had yet to form due to the lack of purged fluids. The donors remained in this state until the winter snow coverage. After the spring melt, overwintered donors emerged in the Advanced phase (227-262 days, 1191-1471 ADD). Donors appeared to have retained some soft tissue volume, particularly in the upper arms and legs. Skin had a glossy leathery appearance with patches of brown, beige, black and orange discolouration. Large areas of white-yellow adipocere had also visibly formed on the hip, inner legs and shoulders of Donor 3 (see figure S25 of Ribéreau-Gayon et al. (2023)). Only a few sparse fly larvae, beetle larvae and adult flies were present on the body or body-soil

interface. At this point, a dark CDI had finally formed with liquid and semi-solid fatty deposits adjacent to the body. Donor remains continued to further desiccate and lose volume into the Dry phase (> 300 days, > 3000 ADD), however bone exposure was overall minimal. A darkened CDI with semi-solid fat deposits remained throughout the final phase.

Soil chemical analyses

Relative to further distances (+50, 50, 100 cm), mean DOC values significantly increased ($p < 0.05$, Tukey, **Table S3.2**) in the A-horizon of soils collected at +20, 0 and 20 cm during the Active, Advanced and Dry phases of donors deposited during warmer months (**Figure 3.1**). The A-horizon close to donors deposited in colder conditions similarly experienced a significant ($p < 0.05$, Tukey, **Table S2**) increase in DOC, but the effect was delayed until the Dry phase. The greatest increases were produced at 0 cm during the Active (5.30 ± 2.92 $mgC \cdot gSoil^{-1}$) and Dry (4.17 ± 1.85 $mgC \cdot gSoil^{-1}$) phases of decomposition for donors deposited in warm and cold months, respectively. Decomposition posed a limited effect on the B-horizon. Highest values were 2.05 ± 1.60 $mgC \cdot gSoil^{-1}$ (warm, 0 cm, Active) and 2.30 ± 1.92 $mgC \cdot gSoil^{-1}$ (cold, 0 cm, Dry), which were not significantly different from adjacent samples. Based on these results, values from distances of +20, 0 and 20 cm for all proceeding analyses were averaged into a mean CDI value for each decomposition phase. The remaining distances (+50, 50, 100 cm) across all phases were then averaged into a single Non-CDI reference value. Findings from the A-horizon are presented as the main results, whereas results for the B-horizon are included within the supplementary material. This was done to better distinguish the spatial effect of body decomposition, to highlight its greater effect on the A-horizon, and to ease data visualization.

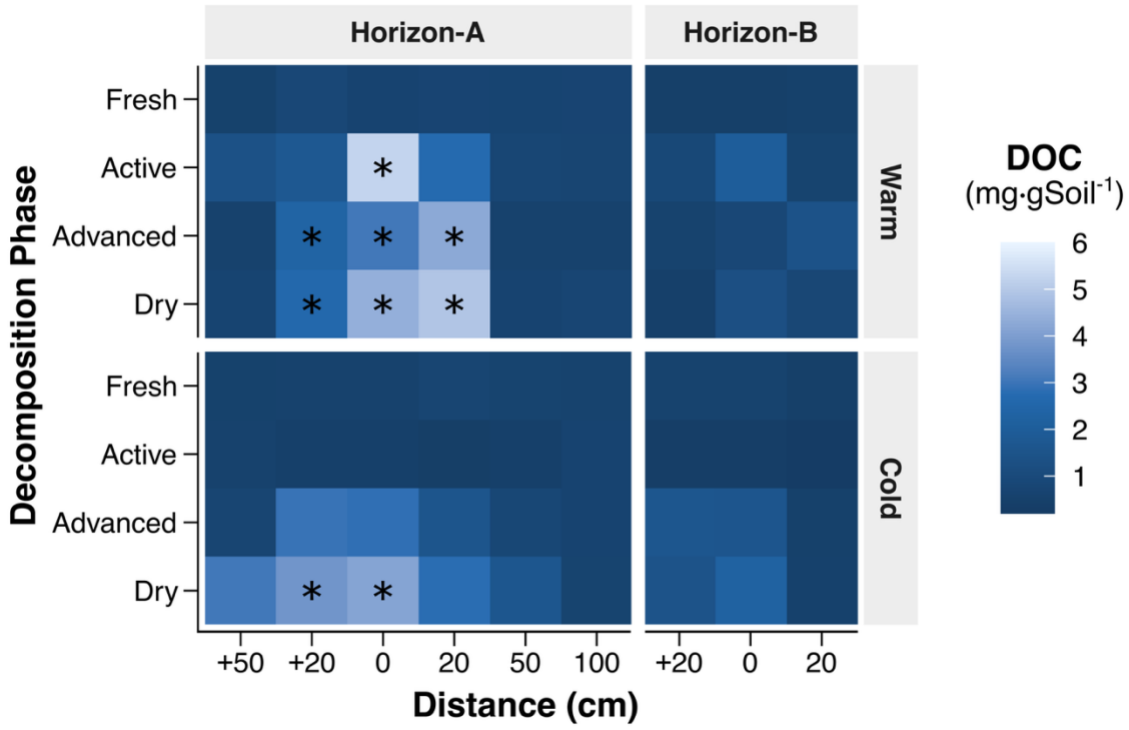

Figure 3.1 – DOC concentration from soils collected around decomposing human donors. Mean DOC levels recorded in soils collected at varying lateral distances and horizons throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Distances denoted with '+' indicate uphill samples, while all other values represent downhill positions. Significant differences ($p < 0.05$, Tukey) between sampling distances within each decomposition phase are represented by an asterisk (*).

Relative to Non-CDI samples (> 20 cm), body decomposition led to an increase in CDI total C and total N (**Figure S3.5**) within the A-horizon. Donors deposited during warmer months produced a significant 81% ($p < 0.001$, Dunnett) and 55% ($p = 0.003$, Dunnett) increase in total carbon during the Advanced and Dry phases, respectively. Total N similarly increased during the Advanced (+59%, $p = 0.004$, Dunnett) and Dry (+83%, $p < 0.001$ Dunnett) phases. CDI soil from donors deposited in cooler conditions had significantly lower %C (-50%, $p = 0.013$, Dunnett) and %N (-48%, $p = 0.019$, Dunnett) during the Fresh phase. Both %C and %N increased with subsequent phases, but these changes were non-significant (**Figure S3.6**). The relative changes in %C and %N resulted in an overall increase in the C:N (**Figure 3.2A**). The mean C:N in the A-

horizon of donors deposited during warmer months significantly increased by 43% during Active decomposition ($p < 0.001$, Dunnett). For donors deposited in colder months, the C:N noticeably, but non-significantly, increased by 36% during Advanced decomposition. No significant changes were detected in total C, total N and C:N within the B-horizon (**Figure S3.6**, **Figure S3.7**).

CDI soil from the A-horizon became gradually enriched in ^{15}N and ^{13}C throughout decomposition **(Figure 3.2B**). $\delta^{15}N$ in CDIs of donors deposited in warmer months significantly increased by 125% ($p = 0.001$ Dunnett) during the Advanced phase and 161% ($p < 0.001$, Dunnett) during the Dry phase. Donors deposited in cooler conditions also produced a significant 152% increase, but only in the Dry phase ($p < 0.001$, Dunnett). Overall A-horizon $\delta^{15}N$ values ranged between 2.51 ± 0.413‰ and 8.02 ± 1.25‰ over the course of decomposition. Soils from the B-horizon presented slightly higher $\delta^{15}N$ values that ranged between 4.63 ± 0.98‰ and 8.05 ± 0.65‰ (**Figure S3.7B**). Within this range, only the Dry phase for cold deposited donors was found to be significantly greater than all other respective decomposition phases (Fresh $p = 0.004$, Active $p = 0.011$, Advanced $p = 0.009$, Tukey). A small, but significant ($p = 0.007$, Dunnett), 4% increase in ^{13}C was also detected in the A-horizon during the Advanced phase of decay for donors who were placed in warmer seasons (**Figure 3.2B**). $\delta^{13}C$ in the B-horizon also generally ranged higher (-27.1 ± 0.151‰ to -25.8 ± 0.309 ‰) than samples from the A-horizon (-27.9 ± 0.304‰ to -26.4 ± 0.384‰), but contrary to $\delta^{15}N$, no significant differences occurred between decomposition phases (**Figure S3.7B**).

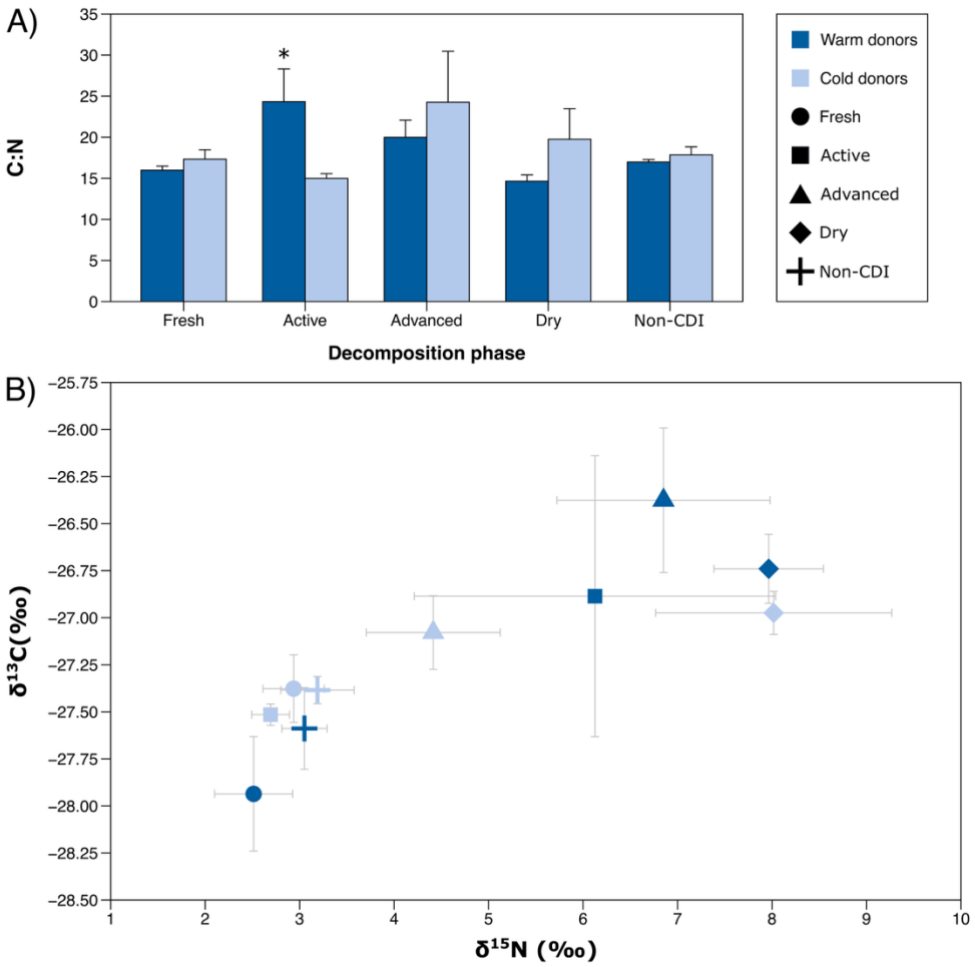

Figure 3.2 – Cabon and nitrogen elemental ratios and stable isotope composition from soils (A-horizon) collected around decomposing human donors.

Mean and standard error of total C:N (**A**) and the relationship between mean $\delta^{15}N$ and $\delta^{13}C$ (**B**) within CDI and Non-CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Significant differences from Non-CDI soils ($p < 0.05$, Dunnett) are indicated by an asterisk (*).

Optical properties in the A-horizon of CDI soils revealed decomposition-related changes in DOM source and chemical characteristics. The percent contribution of terrestrial humic-like PARAFAC components (%C1, %C2) significantly decreased ($p < 0.05$, Dunnett, **Table S3.3**) for all donors during Active, Advanced and Dry decomposition phases (**Figure S3.8**). This was accompanied by both a significant *(*$p < 0.05$, Dunnett, **Table S3.3**) and non-significant increase in

microbial fulvic like (%C3) and protein-like (%C4) components, respectively (**Figure S3.8**). A PCA of mean optical indices (BIX, HIX, FI, S_R) and PARAFAC components (%C1-%C4) from the A-horizon furthermore demonstrated distinct changes over decomposition phase and donor deposition season (**Figure 3.3**). PC1 (48.3% explained variation) was negatively correlated with S_R (r^2 = -0.885, p < 0.001) and %C4 (r^2 = -0.851, p = 0.002), and positively correlated with %C1 (r^2 = 0.807, p = 0.005), HIX (r^2 = 0.741, p = 0.014) and %C2 (r^2 = 0.647, p = 0.043). PC2 (25.8% explained variation) was however mainly defined by a strong positive correlation with FI (r^2 = 0.917, p < 0.001). Fresh and Non-CDI samples from donors deposited during warmer months were initially positioned in the upper right quadrant by the loading of %C2. Samples from the Active phase were then shifted towards the upper left quadrant by the negative PC1 loading of FI, and to a lesser extent, %C3. For the Advanced phase, the negative PC2 loadings of S_R, %C4 and BIX drove a second shift down towards the lower left quadrant. A final shift occurred in samples of the Dry phase, which were driven to the lower right quadrant by the positive PC1 loadings of %C1 and HIX. Soil sampled from donors deposited in colder months demonstrated similar shifts between decomposition phases, but these scores clustered more closely together due to a reduced dispersion along PC2.

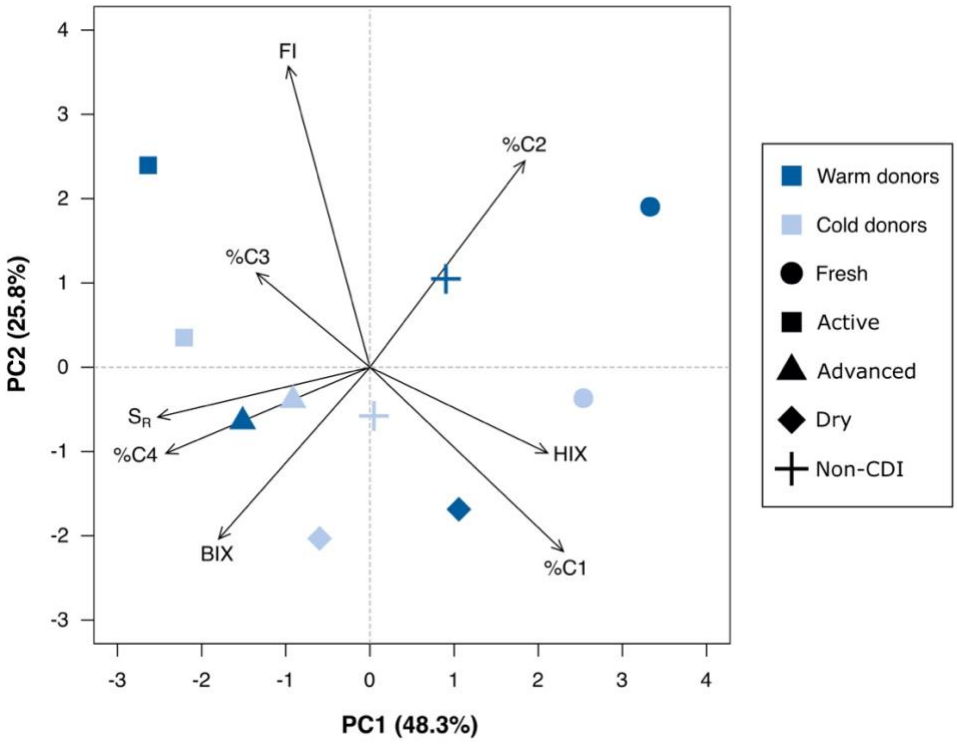

Figure 3.3 – DOM optical properties from soils (A-horizon) collected around decomposing human donors.

PCA biplot of mean values obtained from the optical indices (BIX, HIX, FI, S_R) and PARAFAC components (%C1-%C4) of DOM within CDI and Non-CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions.

Soil bacterial analyses

Bacterial abundance and activity in the A-horizon of CDI soils were greatly affected by body decomposition. Absolute cell counts in the CDIs of donors deposited during warmer months significantly differed relative to the Non-CDI, due to a 137% and 124% increase during the Advanced (p = 0.003, Dunnett) and Dry (p = 0.019, Dunnett) phases, respectively (**Figure 3.4A**). No significant differences in BA were identified in soils of donors deposited during colder months. Mean BR of donors deposited in warmer conditions was significantly different from Non-CDI soils, with peaks occurring during Active (+318%, p = 0.003, Dunnett), Advanced (+735%, p < 0.001, Dunnett) and Dry (+117%, p = 0.049, Dunnett) phases (**Figure 3.4B**). CDIs of donors deposited during colder months also experienced significant increases in BR during Active (+120%, p =

0.005, Dunnett) and Advanced (+112%, p = 0.008, Dunnett) phases. The Fresh phase for cold donors was also found to significantly differ, but this was due to lower respiration rates (-41%, p < 0.001, Dunnett,). Mean BP in CDIs of donors deposited during warmer months (**Figure 3.4C**) also diverged from Non-CDI values due to a significant 98% increase (p = 0.013, Dunnett) during Advanced decomposition. These donors however did not produce any significant differences in soil BGE (**Figure 3.4D**), but there was a perceptible decrease during the Fresh (-19%), Active (-29%) and Advanced (-44%) phases. Soil beneath donors deposited during colder months did however experience a significant 49% decrease (p = 0.004, Dunnett) in BGE during Advanced decomposition.

The B-horizon (**Figure S3.9**) of CDI soils experienced little to no decomposition-related changes in bacterial measures when compared to the Fresh phase. Low mean BR values during the Fresh phase did however result in significant increases during the Active phase (+351%, p = 0.017, Tukey) of donors deposited during warmer months, and the Active (+256%, p = 0.001, Tukey), Advanced (+163%, Tukey, p = 0.008), and Dry (+244%, Tukey, p < 0.001) phases for those deposited in cooler conditions. Although these percent increases appear large, the range of mean BR values for the B-horizon (0.78 ± 0.11 to 8.12 ± 3.32 $\mu gC \cdot gSoil^{-1} \cdot hr^{-1}$) was much less than what was recorded in the A-horizon (0.92 ± 0.12 to 38.55 ± 12.85 $\mu gC \cdot gSoil^{-1} \cdot hr^{-1}$).

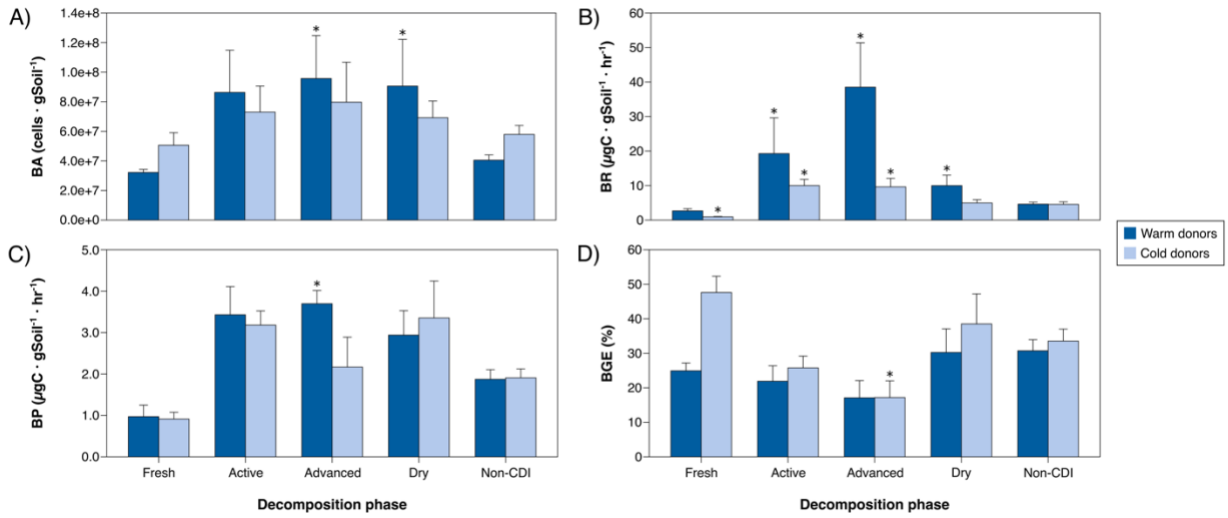

Figure 3.4 – Bacterial abundance and carbon use from soils (A-horizon) collected around decomposing human donors.

Mean and standard error of bacterial cell abundance (**A**), respiration (**B**), biomass production (**C**) and growth efficiency (**D**) within CDI and Non-CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Significant differences from Non-CDI soils ($p < 0.05$, Dunnett) are indicated by an asterisk (*).

A PCA of normalized Biolog EcoPlate™ optical densities revealed differences in substrate utilization patterns across sampling distance, decomposition phase and deposition season (**Figure 3.5**). The effect of decomposition was generally seen along PC1 (34.9% explained variation), which was mostly correlated with carbohydrates and carboxylic acids (**Table S3.4**). Scores for 0 cm and 100 cm samples during early decomposition (Fresh-Active) were clustered towards the left quadrants by a diversity of substrates, but most notably the negative PC1 loadings of carboxylic acids CA2, CA3 and CA5. The scores of 0 cm soils for warm and cold donors began to differ from the starting cluster during the Advanced and Dry phases, respectively. These samples were driven towards the upper right quadrant by the positive PC1 loadings of C6, C1, C10, CA7, A1 and C7. PC2 (19.7% explained variation) marked a distinction between deposition temperatures during the later phases of decomposition (Advanced-Dry). 0 cm soil from donors deposited during warmer months was driven away from all other samples, towards the lower right quadrant, by the negative PC2 loadings of CA1, P2, AA4, AA3, P1 and C9.

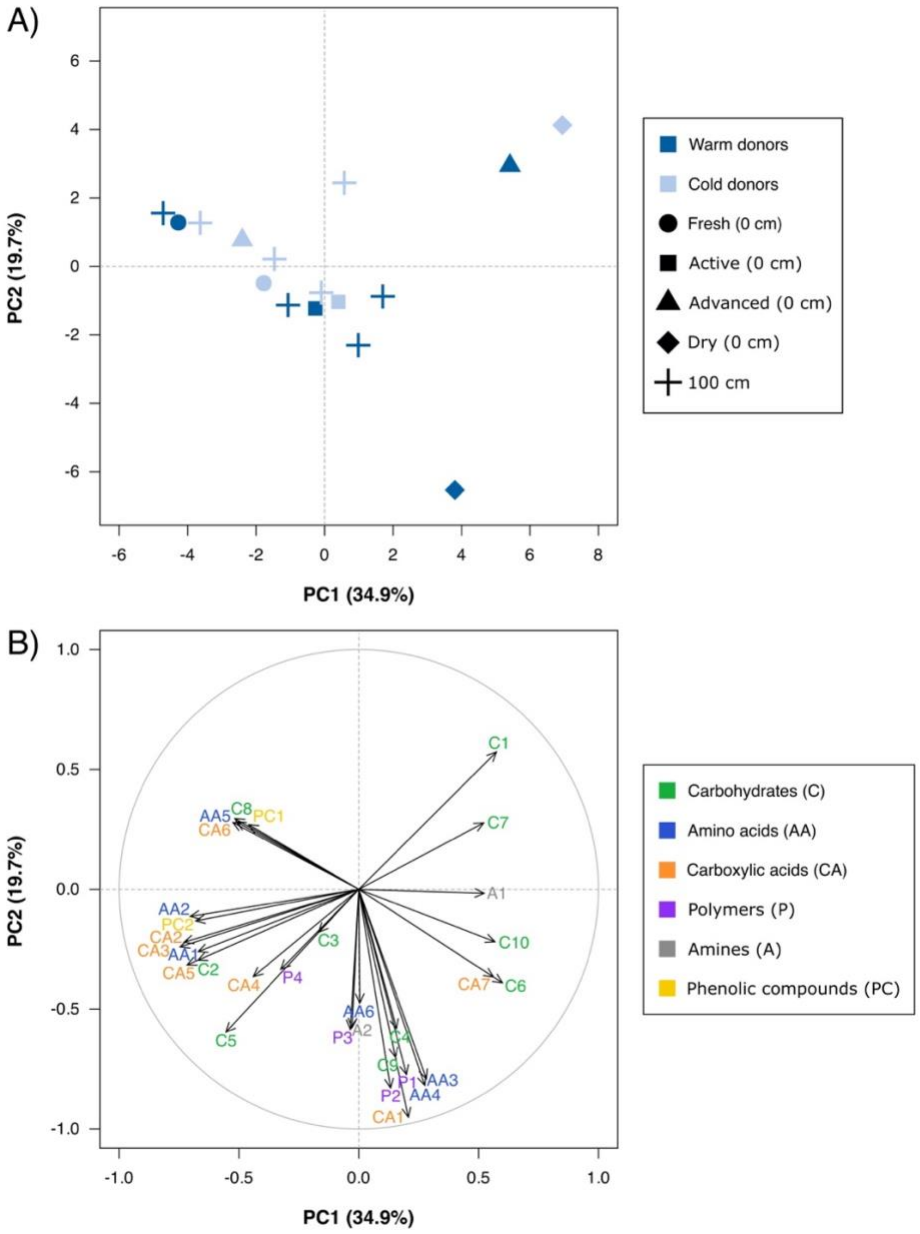

Figure 3.5 – Bacterial metabolic potential from soils (A-horizon) collected around decomposing human donors.

PCA scores (**A**) and loadings (**B**) plots of Biolog EcoPlate™ bacterial substrate utilization patterns obtained from soils collected around (0 and 100 cm) decomposing human donors who were deposited during warm or cold seasonal conditions.

Discussion

As hypothesized, CDIs at REST[ES] posed a disturbance to soil SOM chemistry and bacterial responses. We demonstrated that changes were mostly localized to the A-horizon and within a lateral sampling distance of 20 cm from surface deposited cadavers. The purging of decomposition fluids led to a large pulse in soil carbon followed by nitrogen, which was also accompanied by an enrichment in $\delta^{13}C$ and $\delta^{15}N$. A successive increase in the lability of the DOM pool also occurred, transitioning from terrestrial humic-like to microbial protein-like compounds. The influx of purged fluids also contributed to an increase in soil bacterial cell abundance, while also intensifying bacterial energy use and the preferential metabolism of carbohydrate substrates. We further anticipated that seasonal conditions would affect the timing of CDI biochemical changes. Across all donors, these changes consistently occurred between the Active to Dry phases of decomposition. However, the appearance of these phases and their soil impacts were delayed for donors who were deposited during cooler months. Unexpected tissue preservation from overwintering moreover dampened, but not impeded, soil disruptions.

The progression of gross decomposition and the magnitude of CDI biochemical changes were inconsistent between donors deposited in warm versus cold seasons. Warm donors quickly transitioned through each decomposition phase and presented a typical sequence of putrefaction, insect colonisation, soft tissue loss and desiccation. This led to a large influx of material that produced rapid (Active-Dry, 13-139 days) and significant changes in SOM chemistry and soil bacterial responses. On the other hand, donors who overwintered emerged from the snow with signs of tissue preservation, most notably desiccation and adipocere formation. This is analogous to other human and animal bodies that have been recovered from winter, polar or glacial environments (Hitosugi et al., 2014; Nývlt et al., 2016; Pilloud et al., 2016). Sublimation (i.e. freeze-drying) from cold and dry conditions can encourage tissue dehydration, whereas adipocere can form under the wet and low O_2 setting of melting snow (Pilloud et al., 2016; Piombino-Mascali et al., 2017). Cold deposited donors moreover required a greater number of ADDs to attain each decomposition phase. Cockle & Bell (2017) noted a similar phenomenon in a retrospective study of Canadian forensic cases. Freezing temperatures may destroy

putrefactive bacteria, therefore stalling tissue liquefaction and CDI formation. Upon spring warming, the decimated putrefactive community would then require additional time and ADDs to reestablish itself and resume decomposition (Cockle & Bell, 2017; Pilloud et al., 2016). Nevertheless, cold deposited donors still had a noticeable impact on the soil, although at a reduced magnitude and later timepoint (Advanced-Dry, 227-364 days). Prior to the complete freezing and preservation of tissues, some microbial processes were potentially maintained by the insulating properties of adipose tissues and snow. Accumulated microbial and decomposition by-products could have then been flushed into soil upon warming, further aided by water from snowmelt, thereby creating a CDI under seemingly preserved donor remains. These findings highlight how the CDIs of cold deposited, overwintered and/or preserved donor remains should not be overlooked. Rather, their differences in timing and magnitude should be considered when assessing the environmental impact of body decomposition in temperate regions.

Around 67% of the elemental composition of DOM is made up of carbon. Fluxes in soil DOM are therefore commonly quantified based on dissolved organic carbon (DOC) (Bolan et al., 2011). Hence, we used DOC as an indicator of 1) cadaveric DOM inputs, and 2) the mobility and spatial extent of CDIs. Cadavers supplied large amounts of DOC to CDI soils at REST[ES]. Regardless of decomposition phase or deposition season, all of our observed increases in DOC were constrained to a sampling radius of 20 cm. This matches the limited lateral effect (≤ 30 cm) generated by pig carcasses studied at other eastern Canadian sites (Larizza & Forbes, 2013; Pecsi et al., 2024; Perrault & Forbes, 2016). Maximum concentrations were measured during the Active and Dry phases, respectively, for warm and cold deposited donors. These values fell between levels reported from cadavers placed on loam (1.09 $mgC \cdot gSoil^{-1}$) and silt (10.91 $mgC \cdot gSoil^{-1}$) dominant soils at two Texan HTFs (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017). The broad range of DOC quantities between studies is surely due to a combination of differences in baseline concentrations, edaphic properties, taphonomy and soil extraction protocols. Similar to the Fancher et al. (2017) study, there was also some vertical leaching of cadaveric DOC at lower depths ≥ 13 cm (B-horizon), but the amount was non-significant. The spatially constrained dispersion of DOC, and cadaveric material by proxy, at REST[ES] was likely influenced by a

combination of input location, soil hydrology, mineral adsorption and rapid microbial processing (James & Harrison, 2023).

At REST[ES], we observed an increase in both CDI %N and %C. This has been likewise seen under various vertebrate remains and corresponds well with the influx of DOC and protein-rich matter (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017; Hansen et al., 2016; Keenan et al., 2018). However, the increase in total N lagged in timing and magnitude when compared to C, thus resulting in an elevated C:N. Such a widening in CDI soil C:N is seldomly reported but has been similarly noted by Aitkenhead-Peterson et al. (2012) when comparing DOC to total dissolved nitrogen. Typically, a reduction in C:N occurs from the immense input of nitrogenous compounds generated from the decomposition of soft tissues (Keenan et al., 2018; Macdonald et al., 2014; Quaggiotto et al., 2019). Our contrasting results may be ascribed to the temperate system of REST[ES]. The C:N of human tissue is more akin to the stoichiometric composition of bacteria (~8.1) (Robertson & Groffman, 2015). This balance favors the rapid mineralization of cadaveric organic-N into inorganic ammonium (NH_4^+). Increasing soil pH from NH_4^+ additions can then promote some of its volatilization as ammonia (NH_3) (Dent et al., 2004). The development of an anoxic CDI from purged fluids and excess microbial respiration can subsequently promote denitrification and additional N-losses via N_2, N_2O and NO gases (Fancher et al., 2017; Robertson & Groffman, 2015). These processes could have been intensified at REST[ES] due to previous N-limitations generated during the winter (e.g. from microbial immobilization) and spring seasons (e.g. dilution and transport from snow melt, uptake from growing plants). Rapid mineralization by deprived bacteria potentially led to additional losses of gaseous N-products, thereby lessening the magnitude of cadaveric N-accumulation. This could have contributed to a high soil C:N, as any remaining N would have been overshadowed by the excess carbon derived from donors and pre-existing C-rich leaf litter (DeBruyn et al., 2024). This however necessitates further investigation since we cannot determine which N-cycling pathway was stimulated without measures of organic- and inorganic-N. The total N, total C and resulting C:N however reveals fluxes in SOM turn-over and the availability of energy and nutrients (Ostrowska & Porębska, 2015); aspects that can greatly influence the redistribution of cadaveric material and soil health within HTF ecosystems.

Body decomposition led to a maximum δ^{15}N enrichment of 4.91‰ and 4.83‰, respectively, in soils of donors deposited in warm versus cold seasonal conditions. Such increases in δ^{15}N are the result of three potential processes. The first is the leaching of ^{15}N-enriched decomposition fluids. Human tissues are abundant in ^{15}N (6 to 14‰) due to our high trophic position and urinary excretion of dietary ^{14}N (Bernhardt et al., 2018; Poupin et al., 2011; Tea et al., 2016). Secondly, tissues undergoing decomposition and mineralization will become further ^{15}N enriched from the volatilization of lighter ^{14}N. Leached fluids within the soil can also be subjected to additional enrichment from the continued atmospheric loss of ^{14}N from N-cycling. The drastic shift towards higher δ^{15}N values during Active (warm donors) and Advanced (cold donors) decay, along with the increase in C:N at the same time point, supports our assumption that N-volatilization was occurring. Increases in bacterial cell abundance during the Active to Dry phases of decomposition could have further contributed to enrichment from the presence of ^{15}N-rich microbial biomass (Keenan et al., 2018; Wheeler et al., 2014). Varying degrees of maximum δ^{15}N enrichment have been detected in soils under beaver (10.7‰), salmon (6.8‰) and moose scavenged (2.5‰) carcasses (Bump et al., 2009; Keenan et al., 2018; Wheeler & Kavanagh, 2017). Tissue loss from scavenging or larval feeding can alter the amount of decomposition fluid entering the soil profile (Carter et al., 2007; Heo et al., 2021), and factors like diet, age and disease can moreover affect the starting ^{15}N signature of tissue (Ambrose, 2002; Bernhardt et al., 2018; Tea et al., 2021). It is therefore difficult to distinguish if inter-study differences in δ^{15}N absolute values are due to species, taphonomy and/or microbial N-processing. But given the consistent trend in enrichment, general increases in soil δ^{15}N can be interpreted as an indicator of terrestrial vertebrate decomposition and heightened N-cycling.

We also observed a maximum δ^{13}C enrichment of 1.21‰ in the CDIs of warm donors. The dietary consumption of C4 plants (e.g., maize, sugar) gives modern humans a uniquely enriched ^{13}C signature that can range from -25.26‰ (oral mucosa) to -16.60‰ (hair) (Bernhardt et al., 2018; Bogusiak et al., 2020; Hülsemann et al., 2015). The abundance of ^{13}C derived from human tissues and decomposition fluid was still detectable even upon mixing with depleted soils. Decomposing beaver and salmon carcasses did not comparably create increases in CDI δ^{13}C. In Keenan et al. (2018), the ^{13}C content of beaver tissues was potentially not great enough to

generate a detectable enrichment in surrounding soils, likely because the beavers' herbivorous diets were centered around similar C3 plants that the study site's SOM originated from. The mainly carnivorous marine diet of salmon used in the Wheeler & Kavanagh (2017) study would have provided amply enriched tissues (Wheeler et al., 2014), but there were likely insufficient inputs to enact a change due to the smaller carcass mass. The reduced efflux of cadaveric material from cold deposited donors would likewise explain why a lesser and non-significant enrichment (0.41‰) occurred. $\delta^{13}C$ values of fatty acids extracted from soil have been previously used as supporting evidence that a temporary grave was previously occupied by a known victim and not a commonly buried domestical animal (Bull et al., 2009). Our findings and the lack of reported $\delta^{13}C$ enrichment by other vertebrates shows promise that bulk soil $\delta^{13}C$ can similarly be used as an indicator of decomposition for large C4 eating omnivores, such as humans. In the context of HTFs, stable isotope analyses can moreover be used to track cadaveric inputs and help delineate disrupted zones. The technique may however only be useful for surface or shallow soils since enrichment naturally occurs with depth from the discrimination of heavier isotopes by various biogeochemical processes (Boström et al., 2007; Keenan et al., 2019). Further investigation in a burial setting is therefore warranted.

Fluorescence spectroscopy revealed two major shifts in the chemical composition and characteristics of DOM in CDI soils throughout body decomposition. The DOM pool was first dominated by PARAFAC component C2, whose red shifted emission peak reflected aromatic, hydrophobic and conjugated molecules that are typical of vegetal components (Kothawala et al., 2014; Stedmon & Markager, 2005). This grouping of DOM is highly refractory and is designated as "humic-like" due to the contribution of humic substances (humic acids, fulvic acids, humin) (Fellman et al., 2010). Such DOM fluorophores are highly prevalent in soils from the degradation leaf litter and are representative of the baseline environmental signal. DOM during Active decay was differently characterized by C3 and FI, suggesting a transition to lighter microbially-derived fulvic acids (Gabor et al., 2014). This was unexpected since these types of fulvic-like compounds are typically generated from the transformation of labile precursors, which were not yet detected at this time point (Gabor et al., 2014; Hansen et al., 2016). Spectrofluorometric techniques are unfortunately limited in registering aliphatic labile carbon compounds due to the lack of optically

active aromatic structures (Hansen et al., 2016). However, increases in soil DOC, %C and bacterial activity observed during the same phase, particularly for warm donors, demonstrate that there was a concurrent pulse in microbially favorable labile compounds. The rapid utilization of this labile DOM could have generated fulvic-like compounds through biotic humification mechanisms like selective preservation and the spontaneous polymerization of microbial by-products (Balser, 2004). It is also possible that fulvic-like DOM was also introduced into CDI soils by non-microbial organisms. Housefly and Black Soldier Fly larvae have been shown to enhance fulvic-like DOM fluorescence in animal manure (Cai et al., 2024; Wang et al., 2016). Larval excretions and secretions from growing maggot masses aboveground could have therefore directly supplied fulvic-like DOM into CDI soils. Reduced insect colonization and activity can moreover justify why a smaller shift was observed under cold deposited donors.

A secondary transition in DOM composition was seen during the Advanced and Advanced-Dry phases, respectively, for warm and cold donors. Here, soil DOM became increasingly defined by low molecular weight (S_R), protein-like (C4) compounds that are associated with recent biological activity (BIX). The small size and blue-shifted emission spectra (reduced aromaticity) designated labile characteristics that are linked to hydrolysable amino acids (Fellman et al., 2010). The appearance of labile proteinaceous DOM coincided with the highest levels of soil total N, ^{15}N and bacterial abundance, respiration, and biomass production. This co-occurrence indicates that the growing fraction of proteinaceous DOM was likely a mixture of both cadaveric proteins derived from soft tissues, as well as microbial enzymes and intracellular peptides [45]. A similar proteinaceous optical signature has been detected in CDI soils of pig carcasses that were in a comparable state of Advanced decay (Pecsi et al., 2024). Continued microbial processing of cadaveric inputs throughout the Dry phase, for warm donors, led to the humification (HIX) of DOM compounds into forms that were more akin to terrestrial humic substances (C1), though they still differed from the baseline composition. The accumulation of such compounds in CDIs can theoretically lead to the long-term persistence of chemically distinct SOM, as the chemical stability of humic-like compounds can result in resident times of several years to centuries (Chapin et al., 2002). This persistence could serve as a useful indicator of body decomposition for

forensic applications, but if left unmanaged in HTFs, may inflict unfavorable changes in soil properties (e.g. texture, adsorption, permeability) (Guggenberger, 2005).

CDI soil bacteria adeptly responded to the influx of cadaveric material. The abundance of bacterial cells rose between the Active and Dry phases of decomposition. Majority of these cells are presumed to be exogenous cadaveric- and larval-associated bacteria that were transported during the leaching of fluids (Burcham et al., 2024; Cobaugh et al., 2015). Some additional cells could have arisen through the growth and reproduction of endogenous groups, as rates of biomass production (^{3}H-leucine incorporation) were seen to spike during the same period. But this contribution was likely minimal as shown by the decrease in bacterial growth efficiency (BGE). This dip in BGE is indicative of a preferential allocation of carbon towards energy production (respiration) relative to growth and has been likewise seen in CDI soils at the University of Tennessee's HTF (Cobaugh, 2013). Surges in CDI respiration have been universally reported under various vertebrates (Carter et al., 2007; Keenan et al., 2018; Pecsi et al., 2024; Risch et al., 2020; Stokes et al., 2009) and has usually been accredited to the high energy demands of emerging copiotrophic taxa (Cobaugh et al., 2015; Strickland & Wickings, 2015). The large addition of cadaveric carbon and nitrogen would also be expected to simultaneously promote growth by sufficiently fulling energy and nutrient requirements (Soares & Rousk, 2019; Tate, 2020). That assumption is however dispelled by the observed decrease in BGE. Low BGE values are often a symptom of environmental stress, as microbes are required to divert more energy towards metabolic maintenance (Hu et al., 2022). Abrupt fluctuations in CDI physiochemistry, in addition to heightened resource competition, could be imposing stressors that impede efficient growth. Strickland & Wickings (2015) proposed that a high-quality resource, like a vertebrate carcass, should stimulate SOM stabilization through the assimilation of labile inputs into microbial biomass. According to our findings, this theory may only be relevant to later phases of decomposition when CDI stressors begin to fade, as we only saw improvements in BGE and humic-like DOM during the Dry phase. By examining a combination of soil BA, BR, BP and BGE throughout decomposition, we have revealed a dynamic redistribution of carbon between CO_2 and biomass. Understanding bacterial carbon utilization in CDIs will be imperative for

determining how cadaveric inputs are lost redistributed within terrestrial systems like HTFs or outdoor crime scenes.

Decomposition related changes in Biolog profiles have been identified in the CDI and necrobiome of pig and wildebeest carcasses (Fouché et al., 2025.; Heo et al., 2020; Pechal et al., 2013; Pecsi et al., 2024). To our knowledge, we are the first to use this technique to demonstrate functional shifts in the bacterial community of human CDIs. Bacteria from soil sampled directly beneath donors (0 cm) during the Advanced and Dry phases displayed a metabolic preference for several carbohydrates (C1, C6, C7, C10), D-malic acid (CA7) and phenylethylamine (A1). The general utilization of energy-rich labile carbohydrates would suitably reflect the high energy requirements of copiotrophs and stressed bacterial groups (Pecsi et al., 2024). Certain substrates moreover demonstrate a potential favoritism for cadaveric materials. For instance, the phosphorylated sugar alcohol D, L-α-glycerol phosphate (C10), a known precursor to adipose tissue (Possik et al., 2021), has been a consistently targeted substrate in animal CDIs. Concentrated pulses of fatty residues in soils are uncommon outside the degradation of vertebrate adipose tissue (von der Lühe et al., 2017). We therefore believe that the preferential utilization of substrate C10 is the result of emerging lipolytic bacteria that have been selected by the CDI environment. This resonates with the findings of Howard et al. (2010) who saw an increase in culturable lipolytic bacteria and Group I lipase gene copies in a pig CDI. Cadaver decomposition moreover favored the reduction of N-Acetyl-D-glucosamine (C6) and phenylethylamine (A1). The former substrate is an important constituent of animal connective tissues and bone (Chen et al., 2010), whereas the latter is a neuromodulator and by-product of bacterial fermentation (Checcucci et al., 2021). Both have been notably degraded in other swine CDI and fecal studies (Checcucci et al., 2021; Heo et al., 2020; Pecsi et al., 2024), thus further reinforcing the idea that decomposition selects for bacteria that preferentially metabolize vertebrate-associated substrates. Glucose-1-phosphate (C8) was however unexpectedly not correlated with cadaver decomposition. This substrate is involved in glycogen catabolism (glycogenolysis) (Blanco & Blanco, 2017) and was contrarily degraded in pig studies (Checcucci et al., 2021; Heo et al., 2020; Pecsi et al., 2024). The presence of more favorable compounds in human-specific CDIs could inhibit the metabolism of alternative carbon sources like C8; a

phenomenon known as carbon catabolite repression (Brückner & Titgemeyer, 2002). Repeated human studies are needed to confirm these hypotheses. Overall, Biolog substrate utilization patterns showed that cadaver decomposition altered the metabolic function of the soil bacterial community and led to the preferential degradation of vertebrate-associated compounds. This functional shift has implications both forensically and environmentally. For one, it may enhance the turnover of cadaver-specific compounds, therefore reducing their detection, persistence and suitability as forensic markers. On the other hand, the bacterial preference for cadaveric materials could also impede natural processes within HTFs by neglecting the degradation of vegetal inputs.

Conclusion

To date, the impact of CDIs on soil biochemistry have been poorly characterized in temperate HTFs. The current study illustrates how body decomposition can generate a highly localized disruption in SOM chemistry and bacterial metabolic responses. Cadaver decomposition was found to supply large quantities of labile carbon- and nitrogen-rich compounds to the SOM pool. This in turn coincided with a functional shift in the soil bacterial function, which consisted of heightened energy use and carbohydrate metabolism. Results further suggested that microbial processes may have contributed to both the loss and stabilization of organic inputs respectively through mineralization and humification pathways. These CDI effects were observed across all donors but were noticeably delayed and reduced in magnitude for cold deposited donors. Disruptions in bacterial measures appeared more transient (Active-Advanced phase), whereas chemical characteristics like DOM optical properties and stable isotopes (^{15}N, ^{13}C) remained altered till the end of decomposition (Dry phase). The persistent nature of these chemical changes is promising for their potential use as forensic markers of decomposition. They do however imply that a single donor body may produce enduring disruptions in HTF soils, of which downstream consequences on soil physicochemical properties and nutrient redistribution may ensue. This study contributes to a small but growing body of knowledge on cold climate taphonomy and CDI biochemistry, while also highlighting the need for more long-term monitoring of HTF soils.

Acknowledgements

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Ethics Statement

The use of donated cadavers in this research was approved by the Université du Québec à Trois-Rivières ethics sub-committee of the Anatomy Teaching and Research Laboratory (SCELERA-20–12).

Supplementary material

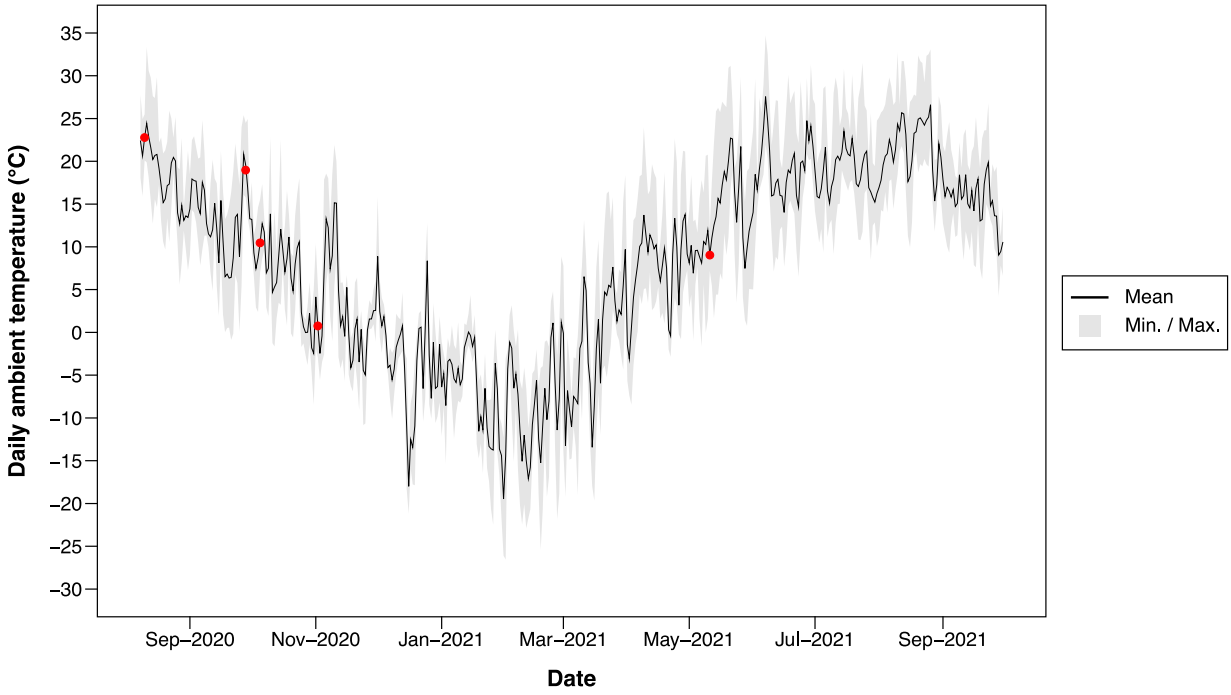

Figure S3.1 – Ambient temperature readings from the REST[ES] facility.

Mean daily ambient temperature recorded from an on-site weather station throughout the duration of the study (10-Aug-2020 to 27-Sep-2021). Shaded area represents minimum and maximum daily temperature ranges. Red dots (•) indicate deposition dates for donors D01 and D02 (10-Aug-2020), D03 (28-Sep-2020), D04 (05-Oct-2020), D05 (02-Nov-2020), and D06 (11-May-2021).

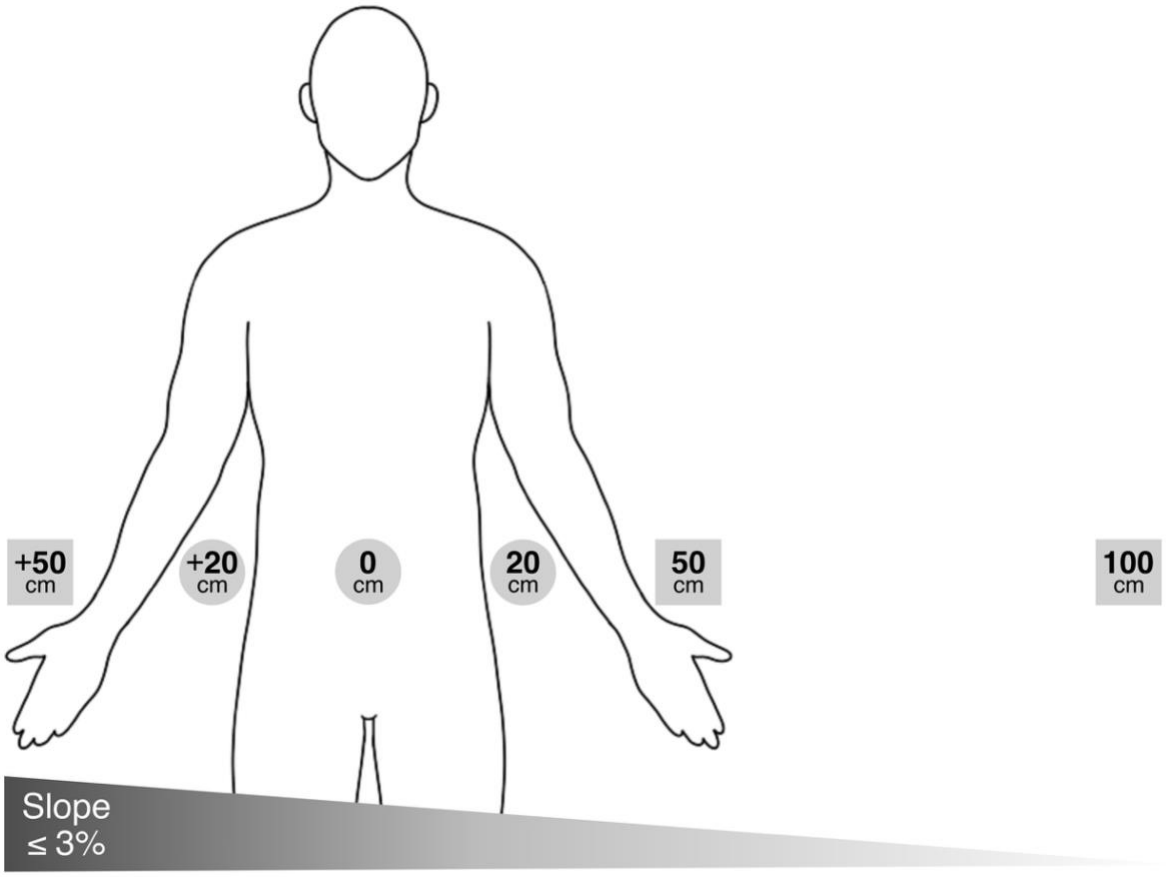

Figure S3.2 – Soil sampling design of the REST[ES] human decomposition study.

Soil cores were collected around decomposing human remains surface deposited at the REST[ES] facility. Distances denoted with '+' indicate uphill samples, while all other values represent downhill positions. Based on the spatial outcomes of DOC levels, all distances indicated by a circle (+20, 0, 20 cm) were averaged into a CDI value for each respective decomposition phase. The remaining distances (+50, 50, 100 cm), indicated by a square, were averaged across all decomposition phases into a single "Non-CDI" reference value. The A-horizon was analyzed across all sampled distances. The B-horizon was only analyzed for CDI (circle) samples.

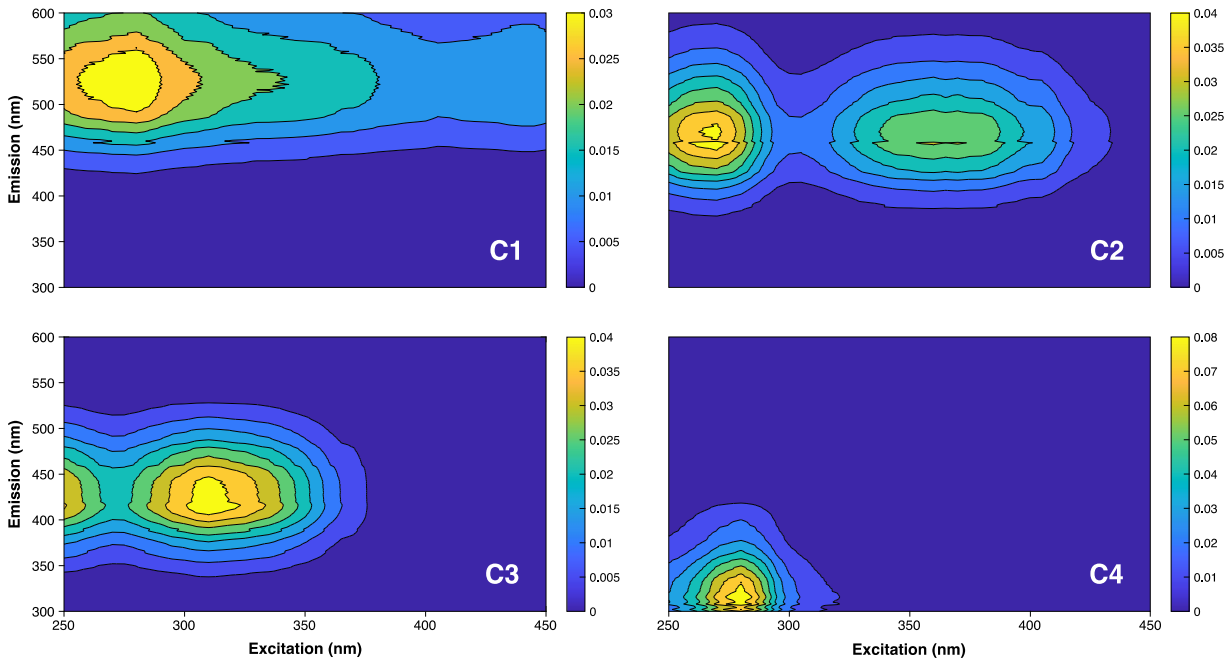

Figure S3.3 – DOM fluorescent fingerprint of validated PARAFAC components (C1-C4) generated from soils collected around decomposing human donors.

Components were identified as terrestrial humic like (C1, C2), microbial fulvic-like (C3) and protein-like (C4).

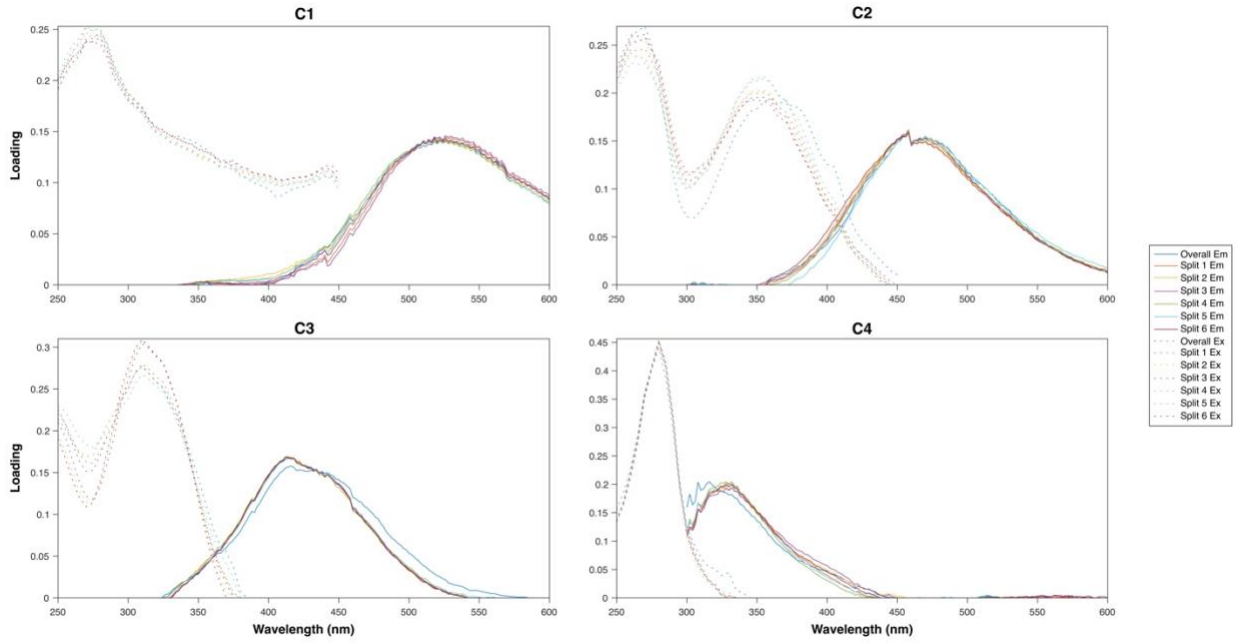

Figure S3.4 – PARAFAC split-half analysis of DOM fluorescence spectra generated from soils collected around decomposing human donors.

Excitation spectra (solid lines) overlaid with emission spectra (dotted lines) for each of the six data subsets generated during the split-half analysis of a four-component (C1-C4) PARAFAC model.

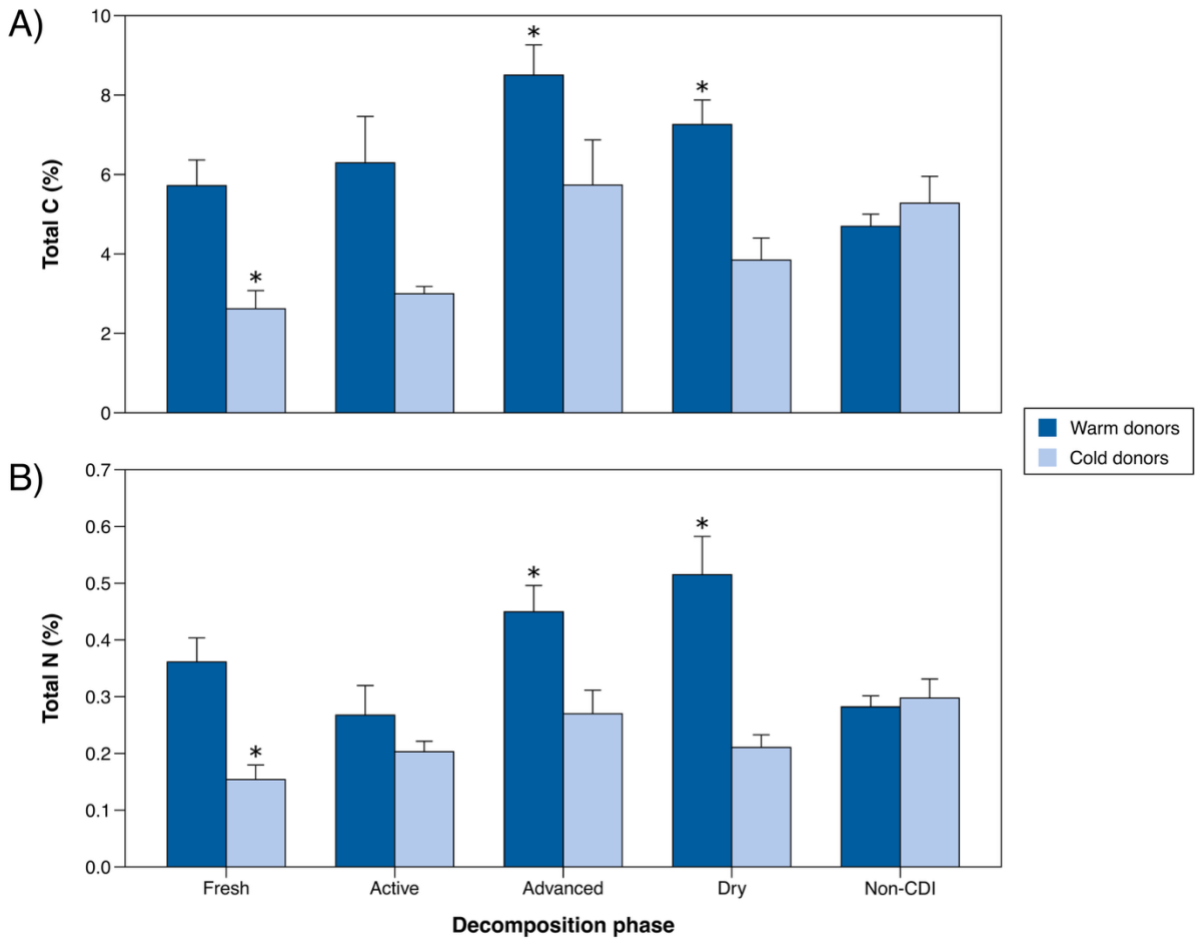

Figure S3.5 – Total elemental carbon and nitrogen from soils (A-horizon) collected around decomposing human donors.

Mean and standard error of total carbon (**A**) and nitrogen (**B**) within CDI and Non-CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Significant differences from Non-CDI soils ($p < 0.05$, Dunnett) are indicated by an asterisk (*).

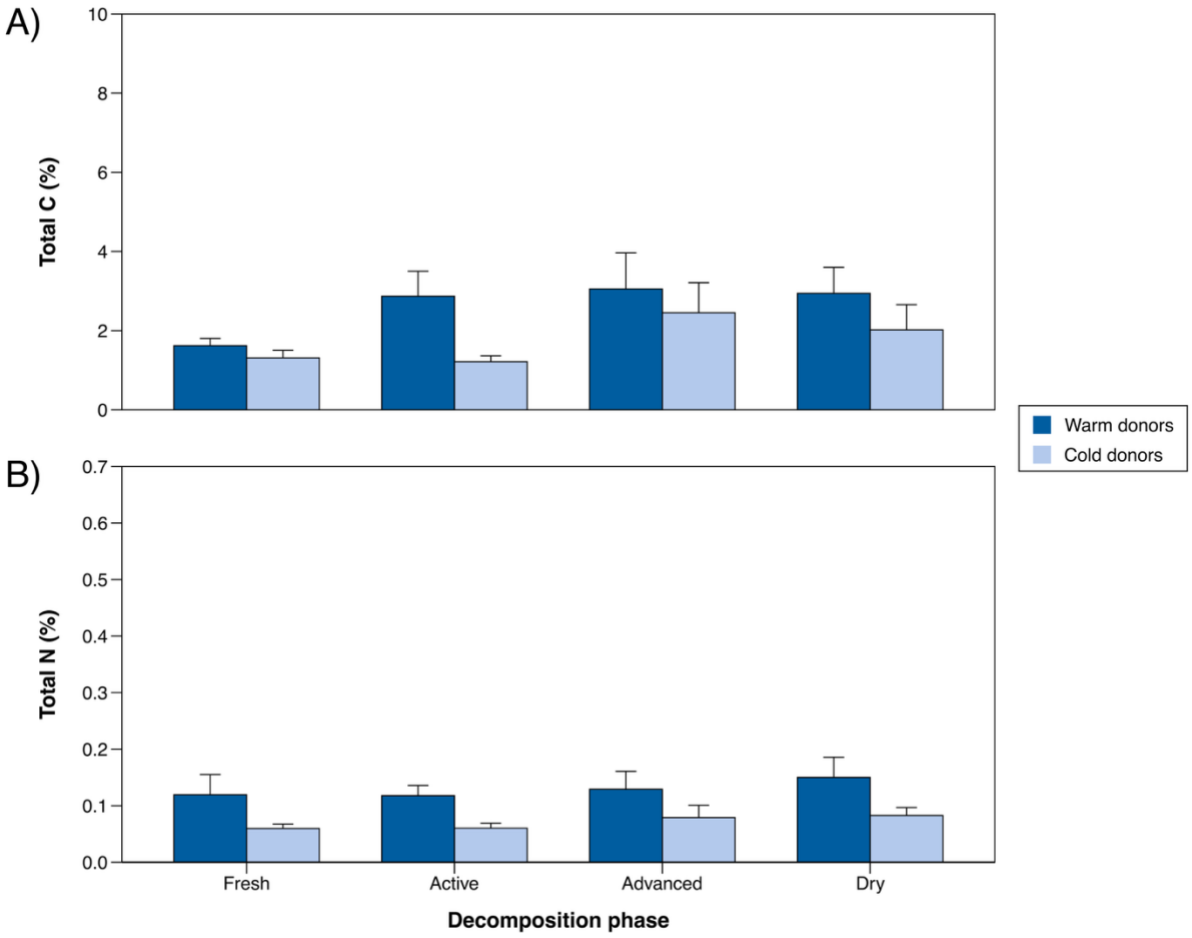

Figure S3.6 – Total elemental carbon and nitrogen from soils (B-horizon) collected around decomposing human donors.

Mean and standard error of total carbon (**A**) and nitrogen (**B**) within CDI soils collected throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. No significant differences were detected between decomposition phases. Scaling reflects Figure S3.5 to facilitate the comparison between soil horizons.

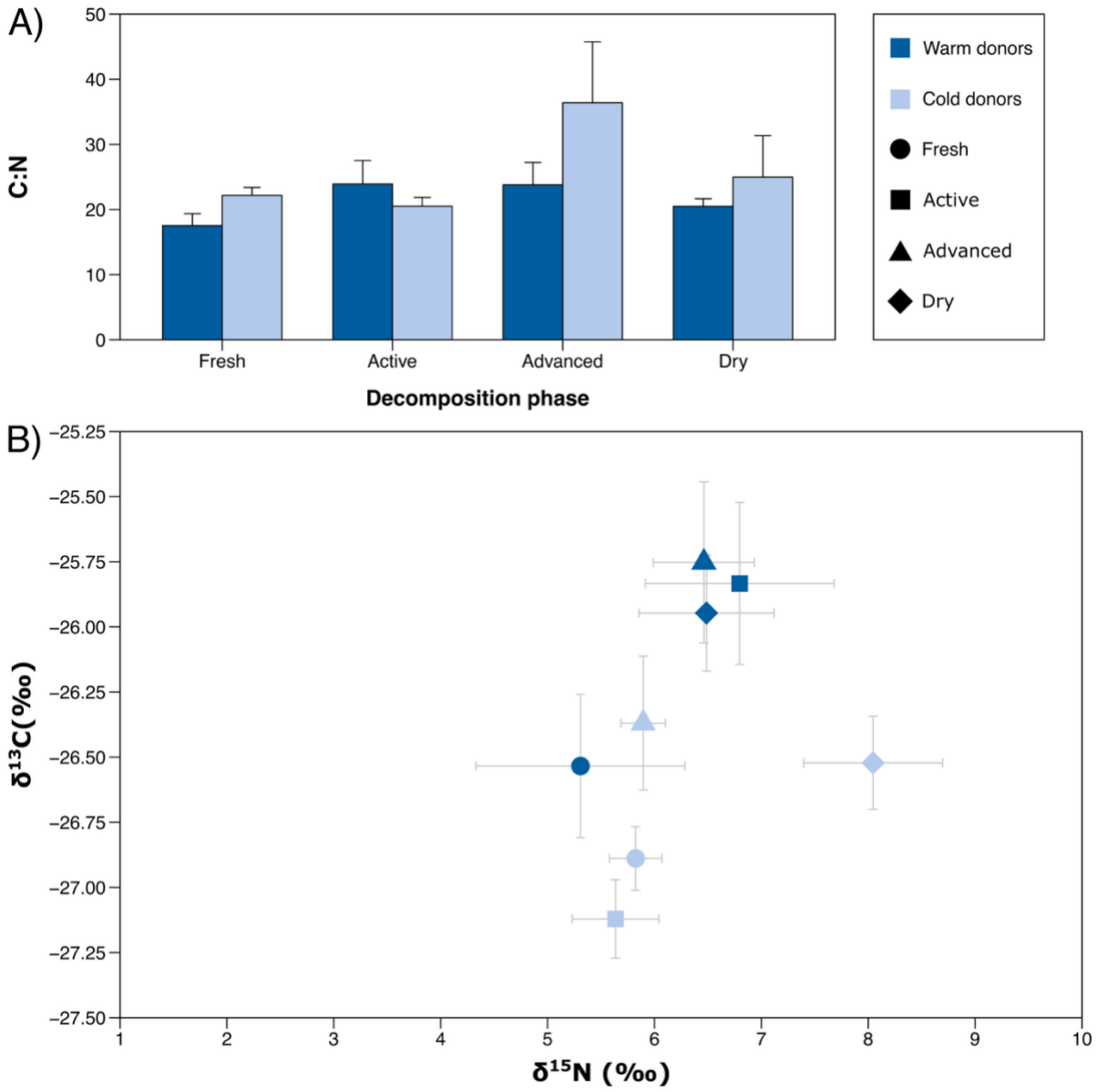

Figure S3.7 – Carbon and nitrogen elemental ratios and stable isotope composition from soils (A-horizon) collected around decomposing human donors.

Mean and standard error of total C:N (**A**) and the relationship between ^{15}N and ^{13}C stable isotope ratios (**B**) in the B-horizon of CDI soils collected throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. No significant differences were detected between decomposition phases in panel A.

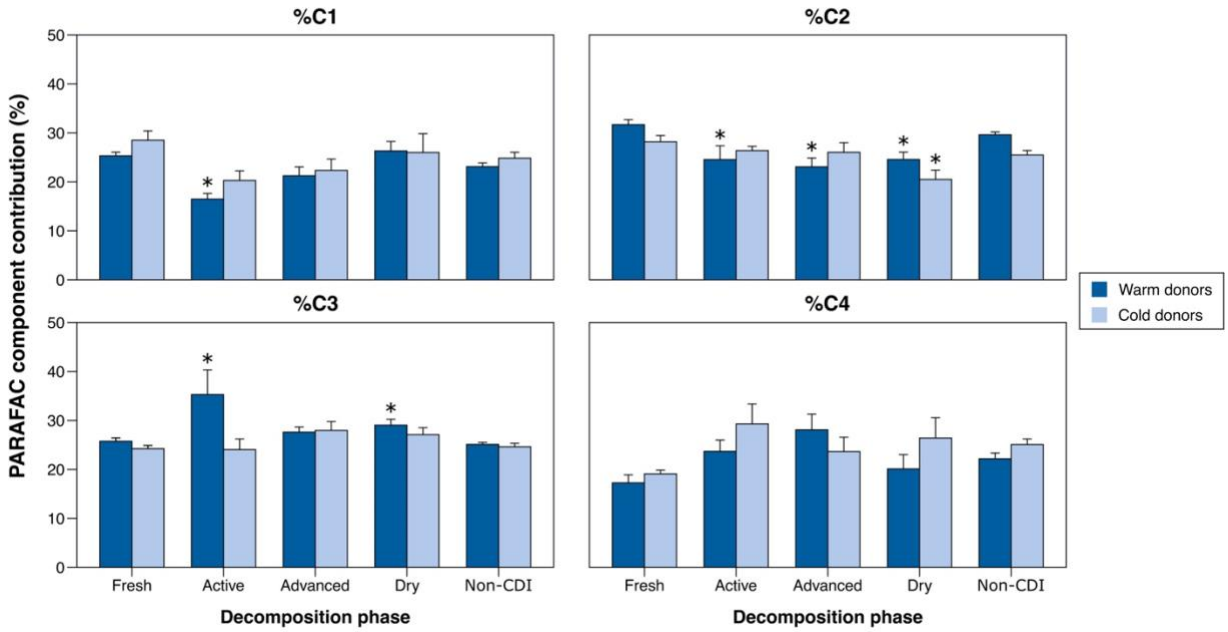

Figure S3.8 – Contribution of PARAFAC components to the total fluorescent signal of DOM from soils (A-horizon) collected around decomposing human donors.

Mean and standard error of the relative contribution of PARAFAC components (%C1-%C4) from CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Significant differences from Non-CDI soils ($p < 0.05$, Dunnett) are indicated by an asterisk (*).

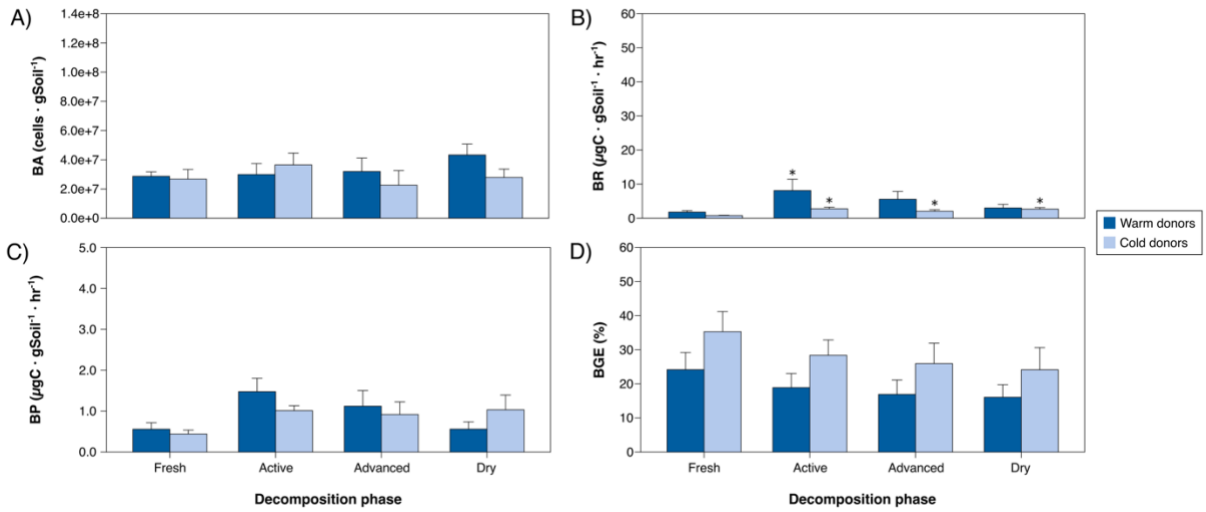

Figure S3.9 – Bacterial abundance and carbon use from soils (B-horizon) collected around decomposing human donors.

Mean and standard error of bacterial cell abundance (**A**), respiration (**B**), biomass production (**C**) and growth efficiency (**D**) in CDI soils throughout the decomposition of human donors who were deposited during warm or cold seasonal conditions. Significant differences between decomposition phases ($p < 0.05$, Tukey) are indicated by an asterisk (*). Scaling reflects Figure 4 to facilitate the comparison between soil horizons.

Table S3.1 – Significant differences ($p < 0.05$) in DOC levels between soils collected around decomposing human donors.

Tukey pairwise comparisons between sampling distances (in cm) within a given decomposition phase following a linear mixed effects model of log-transformed DOC values.

Phase	Season	Soil horizon	Distance comparison	Estimate	SE	*Df*	*t*	*p*
Active	Warm	A	0 – 50	1.71	0.490	40.0	3.49	0.014
Active	Warm	A	0 – 100	1.81	0.490	40.0	3.68	0.008
Advanced	Warm	A	(-50) – (-20)	-1.26	0.400	40.0	-3.14	0.035
Advanced	Warm	A	(-50) – 0	-1.45	0.400	40.0	-3.62	0.010
Advanced	Warm	A	(-50) – 20	-1.73	0.400	40.0	-4.32	0.001
Advanced	Warm	A	(-20) – 50	1.30	0.400	40.0	3.24	0.027
Advanced	Warm	A	(-20) – 100	1.30	0.400	40.0	3.24	0.027
Advanced	Warm	A	0 – 50	1.49	0.400	40.0	3.72	0.008
Advanced	Warm	A	0 – 100	1.49	0.400	40.0	3.72	0.008
Advanced	Warm	A	20 – 50	1.77	0.400	40.0	4.41	0.001
Advanced	Warm	A	20 – 100	1.77	0.400	40.0	4.42	0.001
Dry	Warm	A	(-50) – (-20)	-1.23	0.400	40.0	-3.060	0.043
Dry	Warm	A	(-50) – 0	-1.72	0.400	40.0	-4.30	0.001
Dry	Warm	A	(-20) – 50	1.29	0.400	40.0	3.23	0.028
Dry	Warm	A	0 – 50	1.79	0.400	40.0	4.47	< 0.001
Dry	Warm	A	0 – 100	1.65	0.400	40.0	4.13	0.002
Dry	Cold	A	(-20) – 100	1.62	0.501	38.1	3.24	0.028
Dry	Cold	A	0 – 100	1.73	0.501	38.1	3.45	0.016

Table S3.2 – Significant differences ($p < 0.05$) in PARAFAC component contributions between CDI and Non-CDI soils of decomposing human donors.

Dunnett's post-hoc test between following a linear mixed effects model of log-transformed PARAFAC component contributions (%C1-%C4).

Phase	Season	Horizon	Component	Estimate	SE	*z*	*p*
Active	Warm	A	C1	-0.283	0.074	-3.84	< 0.001
Active	Warm	A	C2	-0.188	0.071	-2.66	0.032
Advanced	Warm	A	C2	-0.277	0.059	-4.68	< 0.001
Dry	Warm	A	C2	-0.206	0.059	-3.48	0.002
Active	Warm	A	C3	0.305	0.056	5.41	< 0.001
Dry	Warm	A	C3	0.143	0.048	3.01	0.011
Dry	Cold	A	C2	-0.221	0.059	-3.74	0.001

Table S3.3 – Significant Pearson correlation coefficients between PCA components and Biolog EcoPlate™ variables generated from soils (A-horizon) collected around decomposing human donors.

Based on PCA in Figure 3.2, which was created using substrate optical densities obtained from 0 cm and 100 cm samples.

	Variable	r^2	*p*
PC1	C6	0.730	0.001
	C1	0.699	0.003
	C10	0.692	0.003
	CA7	0.684	0.003
	A1	0.637	0.008
	C7	0.635	0.008
	CA3	-0.909	< 0.001
	CA2	-0.888	< 0.001
	CA5	-0.873	< 0.001
	AA2	-0.857	< 0.001
	PC2	-0.829	< 0.001
	C2	-0.818	< 0.001
	AA1	-0.817	< 0.001
	C5	-0.675	0.004
	C8	-0.640	0.008
	AA5	-0.630	0.009
	CA6	-0.622	0.010
PC2	CA1	-0.871	< 0.001
	P2	-0.759	< 0.001
	AA4	-0.749	< 0.001
	AA3	-0.724	0.002
	P1	-0.708	0.002
	C9	-0.641	0.007

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Chapter 4 – Disrupting paradise: Sensitivity of soil bacterial responses to labile carrion inputs in a Hawaiian ecosystem

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Abstract

Terrestrial ecosystem health relies on the microbial turnover of soil organic matter (SOM), most of which originates from decaying vegetation. However, climate change and anthropogenic activities are increasing the frequency and concentration of inputs from carrion. The geographical isolation of island ecosystems may render them particularly sensitive to such shifts in SOM inputs. Yet, the response of microbiota to carrion-associated changes in SOM has been understudied in these regions. We aimed to investigate the impact of pig carcass decomposition on SOM-bacterial dynamics in a Hawaiian tropical savanna by using a combination of optical, chemical, metabarcoding, and metabolic analyses. Soils near the carcasses experienced a pulse in organic compounds with semi-labile chemical characteristics. This correlated with the emergence of Bacilli, Clostridia, and Gammaproteobacteria, which were further associated with increases in cell abundance, respiration, and the utilization of carbohydrates and carboxylic acids. When compared to a previous temperate climate study (Quebec, Canada), Hawaiian soils generated a stronger metabolic response despite an equivalent change in SOM chemistry. Our findings demonstrate the sensitivity of Hawaiian soils to carrion inputs and highlight key interactions between SOM and bacteria. These insights will help better position carrion in biogeochemical cycles and inform management strategies for animal mortalities.

Keywords: Vertebrate decomposition, Cadaver decomposition island, Biogeochemical cycles, Soil organic matter, Bacterial metabolism, Bacterial communities, Tropical ecosystem.

Introduction

Soil organic matter (SOM) consists of a mixture of nutrient and energy compounds, whose cycling is essential for maintaining key ecosystem processes such as primary productivity (Horwath, 2015). In terrestrial systems, the majority of SOM originates from the decomposition of plant and animal residues (Chapin et al., 2002). Great efforts have been made towards characterizing vegetal inputs given their abundance and ubiquity across landscapes. Dead animal biomass (carrion) has however, been neglected as a studied SOM resource due to its localized and sporadic distribution (Barton et al., 2024; Parmenter & MacMahon, 2009). Although the information acquired from plant studies is conceptually useful, in practice, carrion likely play a

separate role in SOM dynamics as a result of the unique ecology and composition of animal remains. Unlike plant detritus, carrion offload large quantities of moisture and nutrients that are generated from the degradation of soft tissues and the excretions/secretions of necrophagous species (Barton et al., 2024; Carter et al., 2007; von der Lühe et al., 2017). This is accompanied by an influx of host-associated bacteria largely derived from the purging of gastrointestinal contents (Cobaugh et al., 2015). This leads to the formation of a biochemically distinct "hot spot" known as the Cadaver Decomposition Island (CDI) (Carter et al., 2007). Due to the lack of studies, we do not know what carrion contribute to SOM pools and how it is processed under CDI conditions. Such information will be needed to anticipate potential shifts in SOM cycling resulting from animal mortalities, which are rising in frequency and concentration thanks to climate change (e.g. natural disasters, drought), anthropogenic activities (e.g. livestock mortalities, invasive species) and the loss of scavenger populations (Barton et al., 2019; Newsome et al., 2021).

SOM cycling is influenced by numerous bidirectional interactions between its chemical composition and the metabolic state of microbial communities (Strickland & Wickings, 2015). Composition describes both the quantity and quality of SOM compounds, the former referring to how much SOM is present, and the latter being how easily it is degraded. The bioavailable fraction of dissolved organic matter (DOM) is commonly used to infer information about SOM composition, as these compounds are most prone to microbial processing and cycling (James & Harrison, 2023). Small aliphatic DOM compounds (e.g. carbohydrates, small peptides) are considered highly labile, for their active sites are more readily accessible to microbial enzymes. Large, aromatic and conjugated compounds (e.g. humic substances) are contrarily recalcitrant as they require a greater degree of microbial specialization for their breakdown (Chapin et al., 2002). DOM composition can therefore impose selective pressures on microbial communities, as it dictates substrate accessibility and net energy yields (Kang et al., 2021). The resulting metabolic activity and function of these communities will however control the rate and pathway (e.g.: biomass, stabilization, volatilization) in which DOM, and consequently SOM, are cycled (Gougoulias et al., 2014). Microbes can additionally contribute to the DOM pool via metabolic byproducts and biomass components, as well as modify compound structures through biochemical reactions (Chapin et al., 2002; Horwath, 2015; James & Harrison, 2023). The complex

interplay between DOM and microbes is foundational to energy and nutrient cycling. Characterizing these relationships within CDIs will enhance our understanding of soil's capacity to process carrion inputs, as well as aid in identifying areas of accumulation or loss. In the face of global climate change, this will be imperative for refining carbon budgets and developing environmental mitigation strategies for animal mortalities.

Geographically isolated environments are likely the most vulnerable to carrion-associated changes in SOM cycling. One such region is the islands of the Hawaiian archipelago, which are located more than 3,800 km from the nearest land mass (Harrington et al., 2020). This isolation, paired with the islands' young geological age (≤ 5 million years), has led to high levels of endemism and has limited the evolution of large native mammalian species (K. E. Barton et al., 2021; Olson, 2004; Ziegler et al., 2016). Year-round mild and stable weather conditions have also further reduced the occurrence of natural disturbances (e.g. drastic seasons, storm events) (Harrington et al., 2020). This climatic stability combined with the lack of diversity and historical exposure to animal residues means that Hawaiian microbiota may be sensitive to carrion-driven shifts in SOM composition. Unfortunately, this assumption is unverified since belowground responses to carrion in Hawaii have never been characterized or compared to alternate biogeographic regions. Many Hawaiian islands have been battling invasive populations of feral cats, goats, deer and pigs, whose feeding activities are a recognized threat to local fauna, flora and agriculture (Ikagawa, 2013; Pejchar et al., 2020). These species may be unknowingly causing additional ecological harm through their necromass, especially if Hawaiian soil systems are poorly resilient. Carrion effects on soil biogeochemistry will therefore need to be taken into consideration when drafting future population management and culling policies.

In the present study, we aimed to spatiotemporally evaluate the influence of pig carcass decomposition on SOM-microbial dynamics within soils of a Hawaiian tropical savanna ecosystem. This was achieved by characterizing changes in the quantity (DOC) and quality (optical properties) of the DOM fraction. Microbial responses were moreover examined through soil bacterial community structure, activity, and metabolic potential. Focus was placed on bacteria as they are easily susceptible to environmental disruptions and serve as an important entry-point to the soil food web (Pollierer et al., 2012; Stefanowicz, 2006). Carcass decomposition was

anticipated to create a large pulse in labile DOM that would fuel the emergence of highly active bacterial groups. This was further expected to be accompanied by a functional shift that would favor the metabolism of carrion products. We also aimed to gauge the sensitivity of Hawaiian soil systems by comparing CDI trends to those previously observed in a mixed temperate forest of Québec, Canada (Pecsi et al., 2024). We hypothesized that the relative changes in DOM and bacterial measures would be greater in Hawaii as a result of its isolation. Consistent increases in DOM lability were observed across biogeographic regions, but Hawaiian soils demonstrated dissimilar shifts in bacterial metabolic activity and function. A sequential link between carrion-associated changes in DOM composition, bacterial community structure, and bacterial metabolic responses was also established.

Materials and methods

Carcasses and site description

Three adult pig carcasses (Sus scrofa domesticus) weighing approximately 40 kg each were obtained the morning of March 30th, 2022, from Island Farms LLC (Waianae, Oahu). The pigs were slaughtered in accordance with the Humane Slaughter Act (P.L. 85–765; 7 U S C. 1901) using sharp force trauma to the carotid. The carcasses were wrapped in plastic bags and transported within 2 hrs postmortem to the outdoor decomposition site, where they were surface deposited at least 6 m apart. Carcasses were not covered by anti-scavenger cages since animal activity in the area is known to be minimal (Dibner et al., 2019).

The decomposition site was located approximately 87 m above sea level in the Palolo Valley on the island of Oahu, Hawaii (21° 17′ 27″ N, 157° 48′ 17″ W). The climate and ecosystem of the area were characteristic of a tropical savanna (Kottek et al., 2006). Throughout the study, the site received a total rainfall of 86 mm and experienced an average ambient temperature of 23°C (**Figure S4.1**). The terrain consisted of moderately steep hills (5-20% slope) dominated by guinea grass (*Megathyrsus maximus*), night blooming cereus (*Hylocereus undatus*), aloe (*Aloe spp.*) and carrion plants (*Stapelia gigantia*) (Carter et al., 2023). Soil in the area belonged to the Pamoa series, which consisted of a well-drained, silty-clay (A-horizon, 0-18cm) Torrertic Haplustoll originating from weathered igneous rock (Soil Survey Staff, 2023).

Sampling and soil processing

Soil was collected around the carcasses at increasing lateral distances uphill (-20 cm, -50 cm) and downhill (20 cm, 50 cm, 100 cm, 200 cm) using a handheld soil probe (2.5 X 30 cm). A 0 cm sample was also collected directly underneath the carcasses by gently lifting the legs and abdomen (**Figure S4.2**). One pig (H1) was only sampled to a downhill distance of 50 cm due to a steep cliffside and thick vegetation. Soil cores were individually wrapped in aluminum foil and immediately refrigerated (4°C) at Chaminade University of Honolulu (Honolulu, HI). Sampling was repeated for each visible phase of decomposition: fresh (day 0), bloat (day 1), active (day 4), advanced (day 6), and dry (day 20) (**Figure S4.3**).

Soil cores were emptied into individual aluminum dishes. The organic layer and large pieces of debris were discarded. Remaining soil was air-dried under a fume hood at room temperature for one week prior to being sieved (2 mm) and homogenized. Processed soil was refrigerated (4°C) in sealed plastic bags before being shipped on dry-ice to the Université du Québec à Trois-Rivières (UQTR, Trois-Rivières, Québec, Canada) for further treatment and analysis. The transportation, importation, storage and handling of all soils was carried out in accordance with the regulations and requirements of the Canadian Food Inspection Agency (D-95-26). Samples were immediately refrigerated (4°C) upon arrival at UQTR.

A soil extract was created by shaking overnight at 4°C a 1:40 soil to ultrapure water mixture. Extracts were left upright for a minimum of 30 min. to allow soil particles to settle. Supernatants were collected and mixed to a final concentration of 0.001N $NaHCO_3$ to lightly buffer the samples and replicate the ionic strength of soil systems (Wickland et al., 2007). Buffered extracts were filtered using pre-combusted (500°C, 4 hrs) GF/F (0.7 μm) or GF/D (2.7 μm) glass microfiber filters for chemical and bacterial analyses, respectively. Leftover air-dried soil was weighed and further oven dried at 105°C for 24 hrs. to correct for residual moisture content.

Chemical analyses

DOM quantity was inferred from the concentration of dissolved organic carbon (DOC) in acidified (HCl, pH < 2) GF/F filtrates. This was measured on a Sievers M9 portable TIC/TOC analyzer

(GE Analytical Instruments, Boulder, CO). Instrument performance was verified against a five-point potassium hydrogen phthalate calibration curve. Samples were run in triplicates with an accepted standard deviation of ≤ 10%.

Distinct absorbance and fluorescence signals are produced depending on the underlying molecular structure of the DOM compounds. These structures can vary as a function of source and biological processing, as well as reflect the level of lability (D'Andrilli et al., 2022). Optical properties of GF/F filtrates were therefore used to evaluate the quality of DOM compounds. Fluorescent excitation-emission (EEM) spectra were created on a Carey Eclipse Fluorescence Spectrometer (Agilent, Santa Clara, CA) using a 1 cm glass cuvette. EEMs were measured between excitation (Ex) wavelengths of 230-540 nm and emission (Em) wavelengths of 300-600 nm, respectively, at 5 nm and 2 nm intervals. EEMs were corrected and analyzed using the dr.EEM 0.6.3 toolbox (Murphy et al., 2013) for MATLAB 2024a (Mathworks, Natick, MA). Spectra were corrected for instrument biases using manufacturer provided files. Inner filter effects were also removed by using absorbance spectra (200-900 nm) obtained from a Carey 100 UV-VIS Spectrophotometer (Agilent, Santa Clara, CA) and a 1 cm quartz cuvette. EEM values were normalized to Raman units (R.U.) using the area under the Raman scatter peak of ultrapure water.

EEM- and absorbance-based optical indices were calculated as described in Hansen et al. (2016). The biological index (BIX) served as an indicator of labile DOM produced from recent biological activity (Huguet et al., 2009). The humification index (HIX) represented large, aromatic DOM compounds associated with humic substances (Zsolnay et al., 1999). The fluorescence index (FI) was used to discern between microbial and terrestrial sources (Cory & McKnight, 2005) and the spectral slope ratio (S_R) inversely corresponded to DOM molecular weight (Helms et al., 2008). A total of 95 EEMs were also fitted to an existing Parallel Factor Analysis (PARAFAC) model that was previously developed during the Québec pig study (Pecsi et al., 2024). PARAFAC is a multiway statistical technique capable of decomposing complex 3-dimensional EEMs into individual florescent components, whose peaks can be ascribed to known molecular characteristics and DOM sources (Murphy et al., 2013). The model consisted of four components that represented terrestrial humic-like (C1), microbial fulvic-like (C2), microbial protein-like (C3) and terrestrial

phenol-like (C4) DOM compounds. Components are expressed as their relative percent contribution to the maximum fluorescent intensity (%F_{max}).

Bacterial analyses

Bacterial community composition was characterized by sequencing the V4 hypervariable region of the 16S rRNA gene. DNA extraction was performed using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). A sterile HydraFlock™ swab (Puritan Medical Products, Guilford, ME) was moistened with the kit's CD1 solution and dipped into the soil sample. The soil covered swab head was cut from the handle and placed into a PowerBead Pro tube. The extraction was then performed according to manufacturer instructions. Extracts were stored at -20°C prior to PCR amplification (**Text S4.1**) using 515F/806R primers (**Table S4.1**). Amplicons were sent to the Centre d'expertise et de services Génome Québec (Montréal, Québec, Canada) for library preparation and sequencing on the Illumina NextSeq PE300 platform (Illumina Inc., San Diego, CA). Sequencing data was received as demultiplexed FASTQ files with barcodes already removed. Files were uploaded to the Compute Canada servers for bioinformatics processing following the DADA2 1.8 (Callahan et al., 2016) pipeline (**Text S4.2**). Taxa were assigned to amplicon sequence variants (ASV) according to their alignment (99% identity) with reference sequences in the SILVA ribosomal RNA gene database (Quast et al., 2013).

Absolute cell counts for bacterial abundance (BA) were obtained via flow cytometry. Bacterial cells were extracted and separated from soil particles using a Nycodenz® density gradient (Axis-Shield, Dundee, Scotland) following the protocol of Khalili et al. (2019). Extracts were stained with 2.5X SYBR™ Safe DNA gel (50X in DMSO, Invitrogen, Waltham, MA) in the dark for 15 minutes at room temperature. Cell enumeration was done on a CytoFLEX-S (Beckman Coulter, Brea, CA) equipped with a solid-state laser emitting at 488 nm. Instrument performance and flow rate were verified according to manufacturer instructions using the CytoFLEX Daily QC fluorospheres (Beckman Coulter, Brea, CA). Green and red fluorescence were collected on a log-scale respectively using the FITC (525/40 nm) and PerCP (690/50 nm) channels with an acquisition threshold of 10,000 events. Unstained samples were used to help discriminate autofluorescence,

debris and noise from stained cell populations. CytExpert v2.6 software was used to perform manual gating, which was based off the strategy of Hammes & Egli (2005).

Rates of bacterial carbon respiration (BR) in GF/D filtrates were used to assess levels of metabolic activity. This was measured in the dark, at room temperature, using 4 mL glass vials equipped with PSt5 optical oxygen sensors and a SDR SensorDish™ (PreSens, Regensburg, Germany). Readings were automatically registered every 15 minutes for a maximum of 7 days. The rate of O_2 consumption was determined from the slope of the linear regression of O_2 ($mg \cdot L^{-1}$) over time, which was then converted to the rate of carbon consumption using a respiratory quotient of 0.95 (Hilman et al., 2022).

The metabolic potential of the bacterial community was evaluated using 96-well Biolog EcoPlates™ (Biolog Inc., Hayward, CA), which contained triplicates of 31 carbon substrates (**Table S2.1**) and blanks (water) along with a tetrazolium dye. Wells were inoculated with 120 μL of GF/D filtrates and stored in the dark at room temperature. Purple coloration from substrate reduction was measured by absorbance at 590 nm using a Biotek Synergy H1 microplate reader (Agilent, Santa Clara, CA). Readings were taken twice a day for the first week, then once daily for the following week or until the average well color development (AWCD) of the entire plate plateaued. The optical density of triplicate wells was averaged, blank corrected then normalized to the incubation time at which the AWCD reached 0.5 to account for differences in inoculum density (Garland, 1997). Resulting negative values were considered as zero. Only soil sampled at 0 cm and 200 cm were analyzed due to time and cost constraints. This however still allowed for the comparison of areas that were the most and least affected by carcass decomposition.

Data analysis and statistics

Statistical analyses and graph building were performed in RStudio version 2025.5.0.496 (Posit team, 2025). All statistical tests employed a significance value of < 0.05. A Linear mixed effect model (LME) (*lme4* 1.1-37) followed by a Dunnett test with Bonferroni adjustment were run individually for each decomposition phase to evaluate differences in mean DOC, BR and BA across sampling distances (fixed effect). Pigs were set as a random effect to control for inter-individual variability and values were log transformed to help improve data normality. The 200

cm sampling distance was used as the post-hoc reference value. Principal component analysis (*PCA*, stats 4.5.0) was generated on centered and scaled data to reveal spatial and temporal shifts in DOM composition (DOC and optical properties) and bacterial metabolic potentials (Biolog EcoPlate™ optical densities).

Sample metadata, ASV raw abundances and taxonomic identifications were integrated into a single phyloseq object (*phyloseq* 1.52) for easier data manipulation. Contaminant ASVs present in negative controls were identified and removed using the prevalence-based method of the *decontam* 1.28.0 package. Alpha and beta diversity were assessed on ASV relative abundances by respectively using the Shannon index and a non-metric dimensional scaling (NMDS) of Bray-Curtis dissimilarities (*phyloseq* 1.52). Permutational multivariate analysis of variance (PERMANOVA, *vegan* 2.6-10) was used to identify changes in bacterial class abundances. This was carried out using 999 permutations, pigs as a blocking-factor, and Bray-Curtis dissimilarities of class-level relative abundances. The influence of DOM composition on bacterial community composition, and the latter on bacterial function, were assessed via partial least squares regression (PLS-R) with leave-one-out cross-validation (*plsdepot* 0.2.0). This included Hellinger transformed (*vegan* 2.6-10) abundances of the top eight most variable bacterial classes. A linear regression (*stats* 4.5.0) of PLS-R scores from the first component was additionally conducted for additional dimension reduction and to better view correlation between the overall patterns in explanatory and response variables.

Biogeographic comparisons were made between data from the advanced phase (Hawaii study) and the summer period (Québec study) due to similarities in ambient temperature and the visual state of carcass decomposition. For DOC, BR and BA, only sampling distances of 0 and 100 cm were used to respectively evaluate soils that were most and least affected by decomposition. Their values were control normalized to the mean of their corresponding 200 cm samples to help correct for site-specific baselines. Differences between sampling distance and site were evaluated using LME (fixed effects = distance + site, random effect = pigs) followed by a Tukey pairwise comparison (*emmeans* 1.11.1). RV coefficients were moreover computed (*ade4* 1.7-23) on Procrustes rotated (*vegan* 2.6-10) PCA loadings (*stats* 4.5.0) to compare the degree of shared multivariate structure in DOM composition and Biolog EcoPlate™ profiles.

Results

Dissolved organic matter composition

Carcass decomposition resulted in a significant increase in soil DOC levels, particularly in samples collected within close proximity to the pig carcasses (**Figure 4.1A**). When compared to the 200 cm reference sample, soil at -20 and 0 cm during the Advanced phase of decomposition experienced a respective 3.75-fold (p = 0.006, Dunnett) and 6.44-fold (p < 0.001, Dunnett) increase in DOC. This remained elevated at a 3.57-fold increase (p = 0.030, Dunnett) for the -20 cm samples during the Dry phase of decomposition. There was also a noticeable 2.65-fold increase at 20 cm during the Advanced phase, but this was deemed non-significant likely due a lack of statistical power from the small sample size (n = 3). Control normalized DOC values for 0 cm samples collected from the present study (Hawaii) and the past Québec study were not significantly different from one another, yet both were significantly greater than their corresponding 100 cm samples (Hawaii p = 0.028, Québec p = 0.028, Tukey) (**Figure 4.1B**).

The progression of carcass decomposition was also found to alter the overall patterns in soil DOM composition. Small changes were observed in the relative contribution of PARAFAC components to the total DOM mixture (**Figure S4.4**). A significant (p = 0.020, Dunnett) 1.15-fold decrease in %C1 occurred in the 0 cm samples during the Dry phase of decomposition. In contrast, %C2 between -20, 0, and 20 cm showed a non-significant increase up to 1.45-fold between the Advanced and Dry phases. %C3 however significantly increased up to 1.54-fold in 0 cm (p = 0.028, Dunnett) and 20 cm (p = 0.030, Dunnett) soils during the Advanced phase of decomposition. Component C4 was excluded from analyses since it only contributed < 7% in 12 out of 95 samples. Shifting patterns in DOM composition became more apparent when PARAFAC components were evaluated with optical indices (BIX, HIX, FI, S_R) and DOC quantity using principal component analysis (**Figure 4.1C**). Decomposition-related changes were mainly driven along PC1 (44.9% explained variation), whereas the natural spatial and temporal variation was captured by PC2 (38.5% explained variation). Scores for samples collected during the earlier phases of decomposition (Fresh, Bloat, Active), as well as further distances (-50, 50, 100, 200 cm), were driven towards the left quadrants by the negative PC1 loadings of S_R > %C1 > BIX (**Table S4.2**). Dissimilarly, scores for -20, 0 and 20 cm samples during the Advanced and Dry phases were shifted

towards the right quadrants by the positive PC1 loadings of DOC > FI > %C2 > HIX > %C3 (**Table S4.2**). Samples were dispersed along PC2 mostly by the positive loadings of %C3 > BIX > S_R and the negative loadings of HIX and %C1. A RV coefficient of 0.638 (p = 0.018, 999 permutations) was obtained when the PCA loadings were compared to those generated from the Québec study, thus suggesting a moderate to strong association between the datasets of the two biogeographic regions.

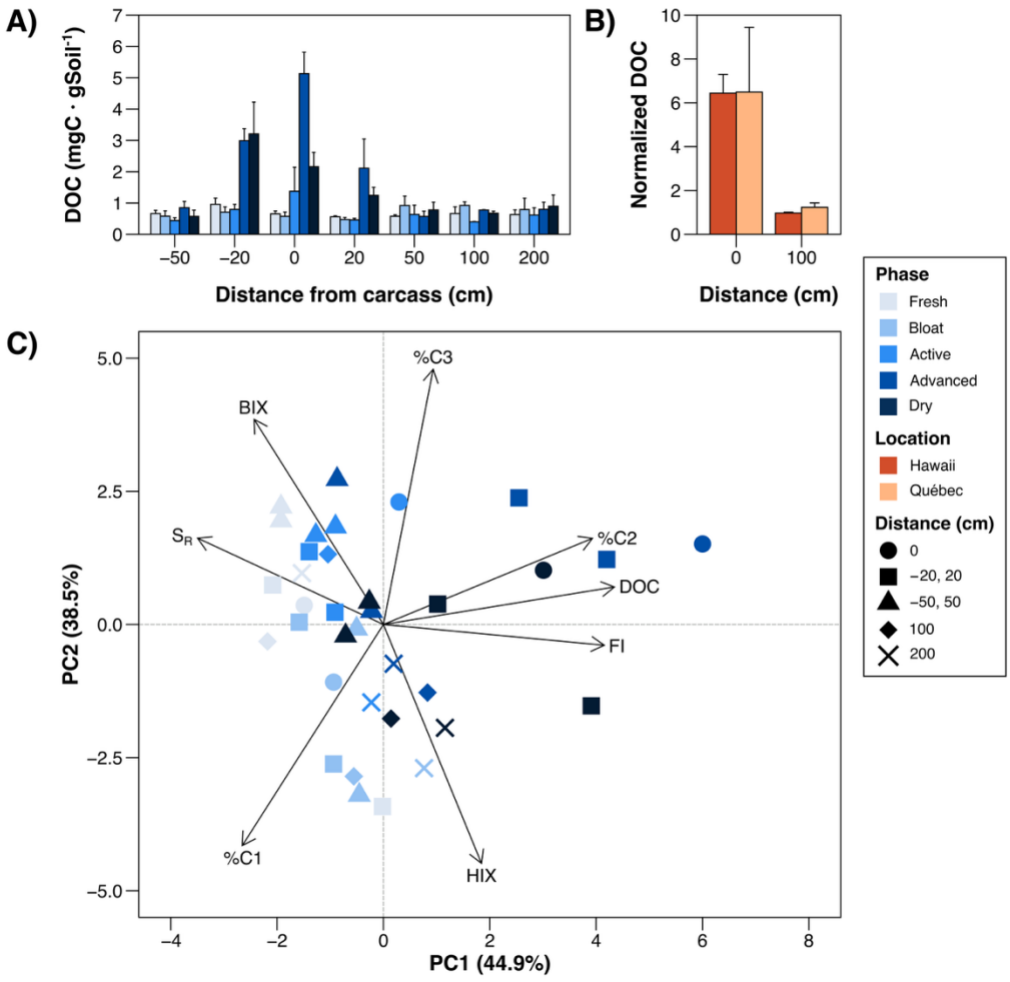

Figure 4.1 – Patterns in soil DOM composition driven by pig carcass decomposition.

(**A**) Mean and standard error of DOC in soil sampled around carcasses deposited in a Hawaiian tropical savanna ecosystem. (**B**) Mean and standard error of control normalized DOC values in soils sampled around carcasses deposited in contrasting tropical (Hawaii) and temperate (Québec) environments. (**C**) PCA biplot of mean DOM optical indices (BIX, HIX, FI, S_R), PARAFAC components (%C1-%C3) and DOC levels detected within soils sampled around carcasses deposited in Hawaii.

Soil bacterial community and metabolic responses

A total of 11,439,032 raw reads were produced from the sequencing of 100 samples, including negative controls. Filtering, denoising and alignment steps using DADA2 resulted in 4,797,235 reads and 48,980 amplicon sequence variants (ASV). Sample decontamination, pruning (removal of 0 read samples), and the exclusion of unwanted sequences (mitochondrial, chloroplastic) returned 3,952,352 reads, equating to 41,603.71 mean reads per sample, and 42,205 ASVs.

Carcass decomposition led to a loss and divergence in CDI bacterial diversity. Community richness and evenness, as evaluated by the Shannon diversity index (**Figure S4.5**), significantly decreased by 1.36- and 1.53-fold during the Advanced phases of decomposition for sampling distances of -20 cm (p = 0.008, Dunnett) and 0 cm (p < 0.001 Dunnett) respectively. A non-significant decrease of 1.14- and 1.26-fold also occurred at -20 cm (Active) and 20 cm (Advanced). A non-metric dimensional scaling (NMDS) of Bray-Curtis dissimilarities further showed a temporal shift in soil bacterial community structure (**Figure 4.2**, 2D stress = 0.193). Soil collected during the Fresh, Bloat and Active phases of decomposition, in addition to all -50, 50, 100 and 200 cm samples, were generally clustered towards the left side of the plot, thus indicating a shared community structure. However, during the advanced phase, bacterial communities from the -20, 0 and 20 cm samples diverged from the starting cluster, as they were positioned towards the right end of the NMDS plot. Soil from those distances but sampled during the Dry phase of decomposition were located between the starting and advanced clusters. A second stage NMDS (**Figure 2**, 2D stress = 0.091) of samples most affected by carcass decomposition (-20, 0 and 20 cm) more clearly demonstrated a divergence in community composition between Fresh-Bloat and Active-Advanced phases of decay. The directionality of the Advanced-Dry phase moreover implies a trajectory back towards the initial community structure.

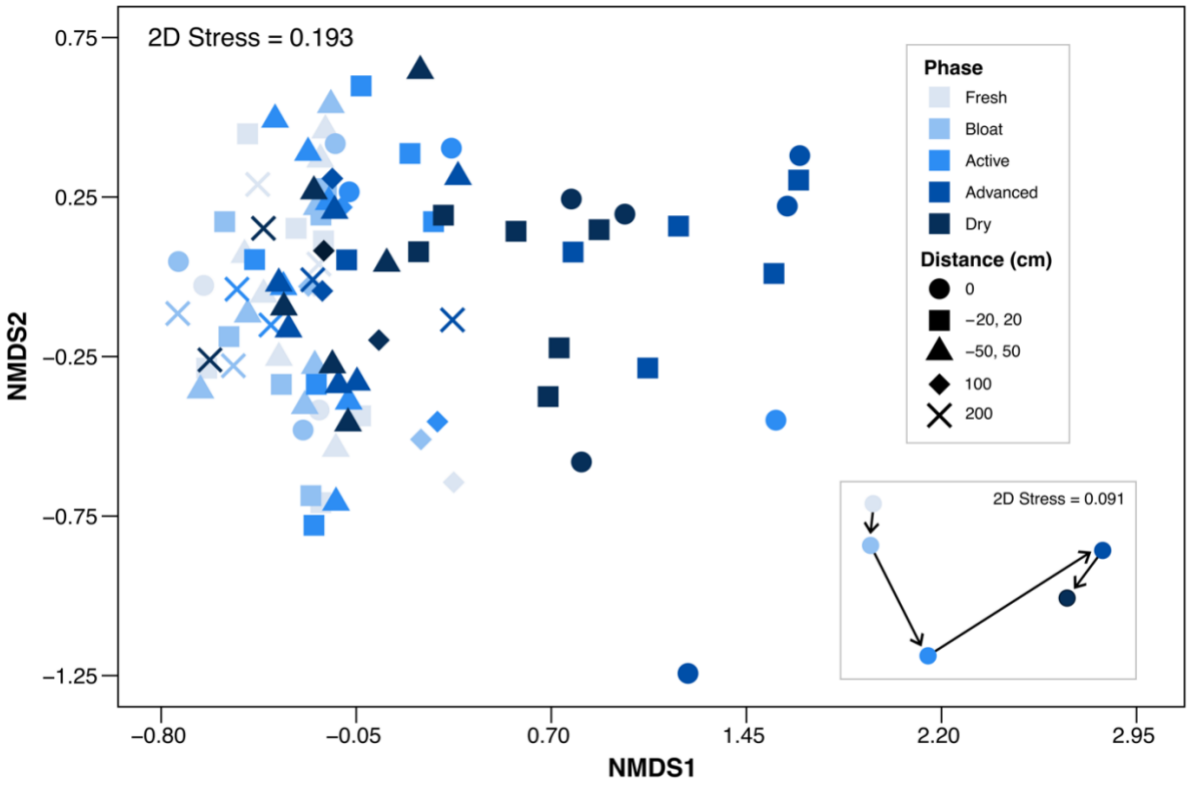

Figure 4.2 – Dissimilarities in soil bacterial community structure around decomposing pig carcasses within a Hawaiian tropical savanna ecosystem.

NMDS plot of Bray-Curtis dissimilarities calculated from the relative abundance of bacterial ASVs. Shapes represent sampling distance, whereas colors represent decomposition phase. Bottom right inset includes a second-stage NMDS demonstrating mean temporal changes in samples most affected by carcass decomposition (-20, 0 and 20 cm).

Examination of bacterial taxa revealed the emergence of newly dominant groups. The native soil community, as found in further sampling distances (-50, 50, 100, 200) and in the Fresh-Bloat phases, were primarily comprised, on average, of Thermoleophilia (29%), Actinobacteria (27%), Alphaproteobacteria (11%), Nitrososphaeria (8%), Rubrobacteria (7%), and Bacilli (4%) (**Figure S4.6**). PERMANOVA supported that decomposition phase did not have an effect on class-level relative abundances in these soil samples. As decomposition progressed into the Active phase, the mean relative abundance of Bacilli increased to 15% in soils sampled at -20, 0 and 20 cm (Figure 3). Bacilli became the dominant group (50%) during the Advanced phase of decomposition. This reduced the relative abundance of all other native classes down to < 26%. At

the same time, Gammaproteobacteria and Clostridia rose in abundance to 7% and 4%, respectively. By the Dry phase, the presence of Bacilli reduced down to an average of 27%, thus allowing Actinobacteria to re-emerge as the dominant class (31%). Gammaproteobacteria (8%) and Clostridia (5%) however remained elevated by the end of the study. The significance of these changes in class relative abundances across the active, advanced and dry phases for -20, 0 and 20 cm samples was confirmed by PERMANOVA (R^2 = 0.341, F = 6.21, p = 0.001, 999 permutations).

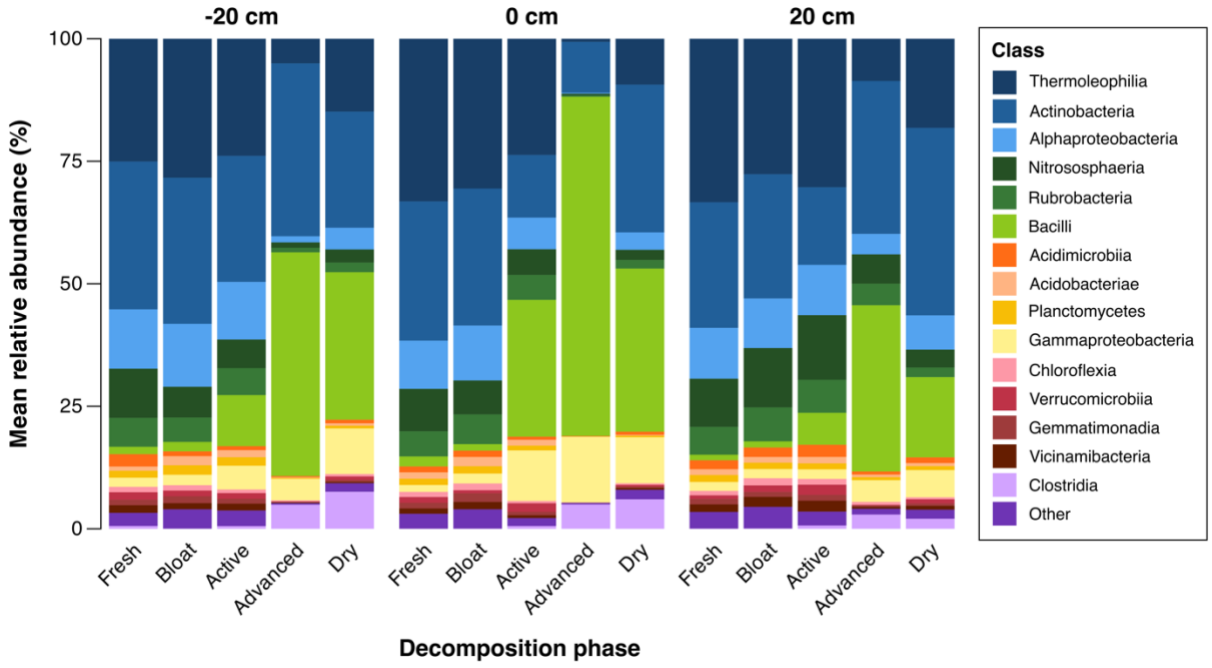

Figure 4.3 – Relative abundance of bacterial taxa, at the class level, in soil most affected by pig carcass decomposition within a Hawaiian tropical savanna ecosystem.

Top 15 most abundant classes are displayed. Remaining classes are grouped as "Other".

Pig carcass decomposition generated heightened levels of bacterial cell abundance (BA) and respiration. Bacterial cell numbers at 0 cm exhibited a (BR). At 0cm, significant 11.62-fold increase in the Advanced phase (p < 0.001, Dunnett) and a 7.74-fold increase in the Dry phase of decomposition (p = 0.004, Dunnett) (**Figure 4.4A**). A notable, but non-significant, 3.83- and 3.11-fold increase also occurred in -20 cm soils during the Advanced and Dry phases, respectively. Control (200 cm) normalized BA values (**Figure 4.4B**) obtained from soils collected under carcasses in Hawaii (p = 0.002, Tukey) and Quebec (p = 0.002, Tukey) were both significantly greater than their respective 100 cm samples. The 0 cm sample from Hawaii was moreover 3.61x greater than

Québec but was deemed non-significant likely from the lack of statistical power. Rates of bacterial respiration (**Figure 4.4C**) in Hawaiian soils significantly increased by 18.4 and 22.7 times, respectively, during Advanced decomposition, at distances of -20 cm (p = 0.002, Dunnett) and 0 cm (p = 0.002, Dunnett). By the Dry phase of decomposition, bacterial cell numbers remained elevated, showing a 9.77-fold increase at –20 cm (p = 0.002, Dunnett) and an 11.6-fold increase at 0 cm (p < 0.001, Dunnett). An anomalous, but significant, 8.92-fold increase (p < 0.001, Dunnett) was also detected at -50 cm during the Bloat phase. Other perceptible but non-significant increases in BR occurred at 20 cm (10.7x) during Advanced decay, as well as -50 cm (6.27x) and 20 cm (5.33x) in the final dry phase. Control normalized BR (**Figure 4.4D**) at 0 cm was significantly greater than 100 cm for both Hawaii (p = 0.007, Tukey) and Québec (p = 0.007, Tukey). Hawaii BR was moreover 3.53-fold greater than Québec values but was once again registered as non-significant.

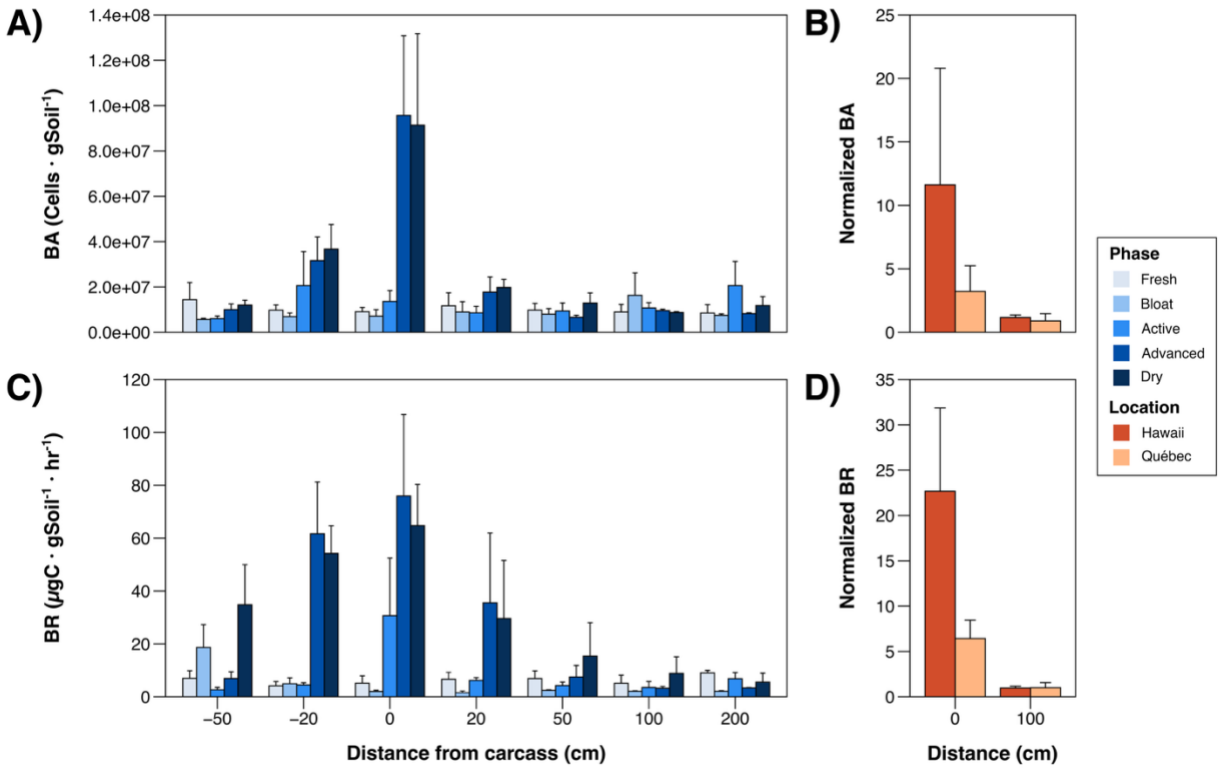

Figure 4.4 – Bacterial cell abundance and activity rates in soils around decomposing pig carcasses.

Mean and standard error of bacterial cell abundance (**A**) and respiration rates (**C**) in soil sampled around carcasses deposited in a Hawaiian tropical savanna ecosystem. Control normalized bacterial cell abundance (**B**) and respiration rates (**D**) in soils sampled around carcasses deposited in contrasting tropical (Hawaii) and temperate (Québec) environments.

The metabolic potential of CDI bacterial communities was seen to diverge with decomposition according to utilization patterns of carbon substrates included within the Biolog EcoPlate™ system (**Figure 4.5**). Shifts driven by decomposition were seen along PC1 (40.1% explained variation), whereas naturally occurring variations were captured along PC2 (17.2%). All scores for 200 cm samples, as well as 0 cm during the Fresh and Bloat phases of decomposition, were distributed within the two left quadrants. These samples were driven by a diversity of substrates, primarily the negative PC1 loadings of P2 > CA7 > A1 > P1 (**Table S4.3**). The 0 cm

samples collected during the active, advanced and dry phases were clustered towards the right quadrants, mostly due to the positive PC1 loadings for CA2 > C5 > C2 > CA5 > CA3 > PC2 (**Table S4.3**). Variation along PC2 were mostly dictated by the positive loadings of AA6 > P1 > C4 > P2 and the negative loadings of C8 > AA2 > P4 > P3 > CA6 > PC1 > C3 > C10 > PC2. The Hawaiian versus Québec PCA loadings resulted in an RV coefficient of 0.297 (p = 0.001, 999 permutations), thus indicating a weak concordance between the substrate utilization patterns.

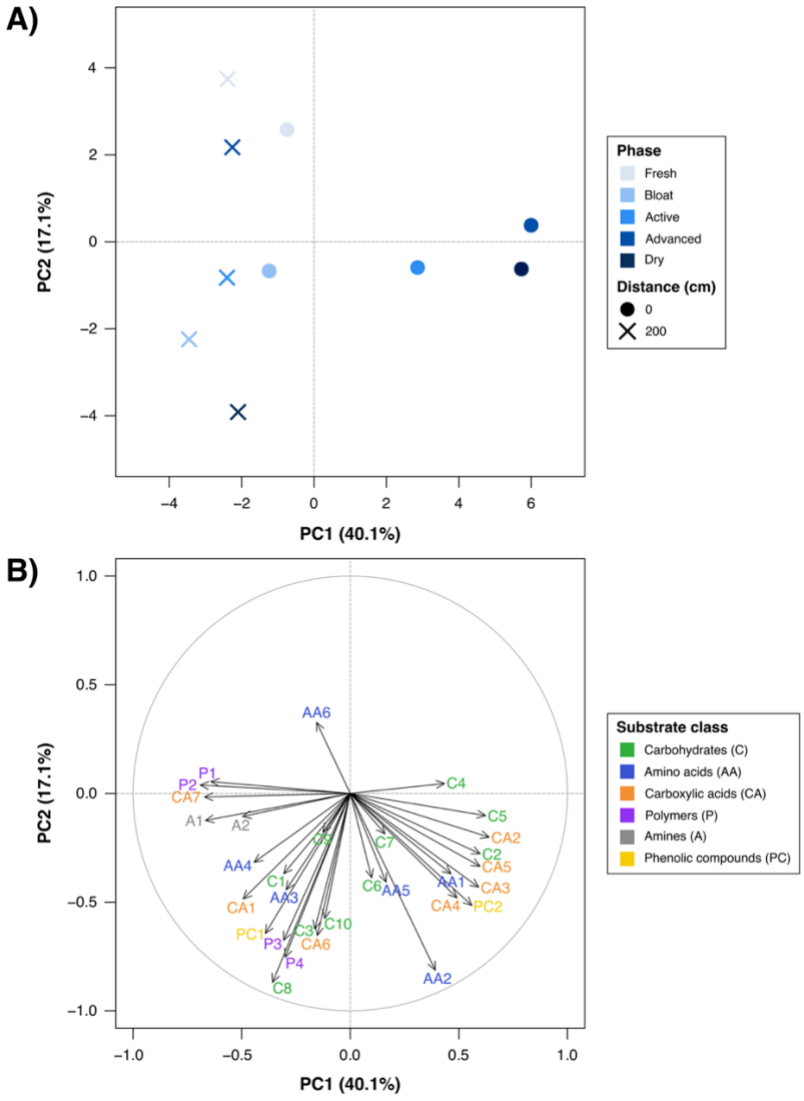

Figure 4.5 – Substrate utilization patterns of soil bacterial communities around decomposing pig carcasses within a Hawaiian tropical savanna ecosystem.

PCA score (**A**) and loading (**B**) plots of mean optical densities developed from the reduction of BioLog EcoPlate™ substrates.

DOM-bacterial relationships

Partial least squares regression (PLS-R) demonstrated associations between carcass-driven shifts in DOM composition and bacterial classes (**Figure 4.6A**). The model's first two components cumulatively explained 83.1% and 65.9% of the variance in the explanatory (DOM) and response (Bacterial classes) matrices, respectively. A $Q^2 = 0.558$ was furthermore produced, thus suggesting a moderate to good predictive ability. In soil most affected by decomposition (-20, 0 and 20 cm for Advanced-Dry), a strong positive correlation is seen between %C2, DOC, FI and Bacilli, Clostridia, Gammaproteobacteria. These three DOM variables were also considered variables of importance to projection (VIP) for both components (**Table S4.4**), along with %C1, which was more correlated with native bacterial taxa Actinobacteria and Alphaproteobacteria. A linear regression of the x (explanatory) and y (response) scores from the first PLS-R component (**Figure 4.6C**) further revealed a very strong positive relationship ($R^2 = 0.863$) between variables. The linear pattern was clearly formed along the CDI's spatiotemporal gradient, where -20, 0 and 20 cm samples from advanced-dry phases were extended towards the upper right corner and all remaining samples were distributed towards the bottom left.

A similar PLS-R approach additionally uncovered that the observed shifts in bacterial classes were also linked to distinct functional changes. This model was produced using Hellinger transformed class abundances from 0 cm and 200 cm samples. The first two components (**Figure 4.6B**) accounted for a total 91.2% and 49.1% of the explained variation respectively for explanatory (classes) and response (function) variables. The model however had a poor predictive ability ($Q^2 = 0.216$), potentially due to the low number of samples used. The emergence of Bacilli (0 cm, Active-Dry) was most positively correlated with C4, C6 and respiration (BR), whereas Clostridia and Gammaproteobacteria were positively correlated with CA2, CA5, C2 and cell abundance (BA). Both Bacilli and Clostridia were deemed VIPs to the first component (**Table S4.5**). Samples least affected by carcass decomposition (Fresh, Bloat, 200 cm) were dissimilarly associated with the VIPs Actinobacteria, Nitrososphaeria and Alphaproteobacteria. Linear regression of x and y scores from the first component (**Figure 64.D**) also demonstrated a strong positive relationship ($R^2 = 0.873$), once again forming along the CDIs gradient.

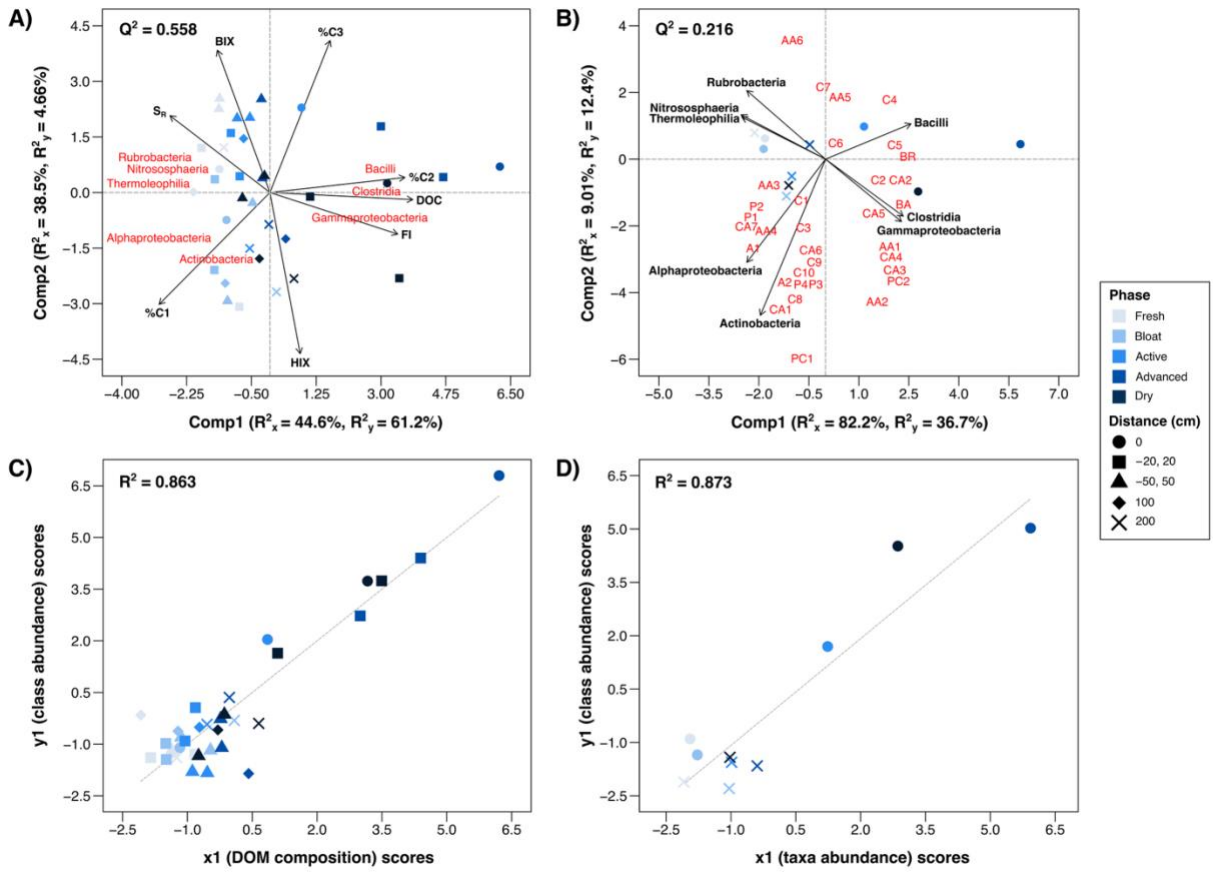

Figure 4.6 – Relationship between DOM and bacterial variables measured in soils around decomposing pig carcasses within a Hawaiian tropical savanna ecosystem.

(**A**) PLS-R biplot linking mean measures of DOM composition (DOC, BIX, HIX, FI, S_R) with mean Hellinger-transformed bacterial abundances of the top eight most variable classes. (**B**) PLSR-R biplot linking the same bacterial class abundances with mean measures of bacterial function (BR, BA, Biolog EcoPlate substrates). Linear regression of explanatory (x) and response (y) variables scores for the first PLS-R component of (**C**) DOM versus bacterial classes, and (**D**) bacterial classes versus metabolic activity and function.

Discussion

Pig carcasses surface deposited in a Hawaiian tropical savanna ecosystem generated significant disruptions in soil DOM-bacterial dynamics. Changes mainly occurred during the Advanced and Dry phases of decomposition, following the purging of fluids, and were spatially isolated to within 20 cm laterally from the carcasses. As expected, carcasses supplied large

quantities of labile microbially-sourced DOM to surrounding soils. This was found to drive a shift in bacterial community structure, consisting of a decrease in diversity from the emergence of dominant taxa. Different taxa were correlated to increases in absolute cell abundance and activity (respiration), while also being dissimilarly associated with a heightened metabolic potential for high-energy carbohydrates or carcass-associated carboxylic acids. Comparisons made with findings from an alternate biogeographic region (Québec, Canada) likewise showed similar shifts in DOM chemistry. Hawaiian soils however, exhibited dissimilar substrate utilization patterns and heightened levels of bacterial cell abundance and respiration.

Relative to the total body mass, only a fraction of a carcass' organic matter ends up leaching into surrounding soils. Putman (1978) estimated that barely 1% of organic matter was transferred between mouse carcasses and their leachates, with the remainder of material either being retained within the carcass or lost through microbial respiration and larval feeding. Although the relative amount of carcass-derived organic matter in leachates may be low, we found that it still managed to produce a substantial spike in soil DOM, as indicated through DOC levels. Indeed, we observed a significant rise in soil DOC during the Advanced phase of decomposition following the purging of fluids. This was in line with other reports of heightened DOC levels in the CDIs of humans, kangaroos, salmon, beavers and pigs (Aitkenhead-Peterson et al., 2012; Chowdhury et al., 2019; Fancher et al., 2017; Heo et al., 2021; Keenan et al., 2018; Macdonald et al., 2014; Wheeler & Kavanagh, 2017). Such sudden shifts in DOM quantity can potentially reshape soil structure and energy reserves (James & Harrison, 2023). However, these quantity-effects would have been temporary and spatially constrained within the scope of our study, as increases in soil DOC were only detected within a sampling radius of 20 cm and were seen to diminish by the final dry phase of decomposition. The high clay content (55%) of the site's soil (Soil Survey Staff, 2023) likely limited the lateral diffusion of fluids even in the presence of a moderate slope, whereas rapid bacterial respiration (as discussed below) could have additionally promoted the loss of DOC as CO_2. The increase in soil DOC levels marked the influx of carcass-associated DOM and essentially defined the spatiotemporal extent of the CDI.

Based on optical properties, we also observed a distinct shift in soil DOM quality. CDI soils (0-20 cm, Advanced-Dry) presented elevated levels of semi-labile microbial fulvic-like (FI, %C2)

compounds, with a modest contribution of labile protein-like DOM (%C3). We thought that carcass decomposition would have supplied a greater amount of labile peptides from soft tissues decay and microbial by-products production (Strickland & Wickings, 2015). We, however, did not expect a greater prevalence of semi-labile fulvic-like compounds, since these only arise from the prior humification (e.g. polycondensation) of labile DOM (Balser, 2004; Gabor et al., 2014; Hansen et al., 2016). Our findings therefore indicated that pre-processed DOM was supplied to our CDI soils. The transformation likely occurred aboveground from the autolytic, microbial and larval breakdown of labile-rich tissues. A similar accumulation in fulvic-like DOM has been seen in human CDIs (see Chapter 3) and from the composting of other animal residues like manure (Wang et al., 2016; Zhang et al., 2012). The fulvic-heavy DOM signature of CDIs differed greatly from that of undisturbed soils (>20 cm, Fresh-Active). DOM in these samples reflected the natural patchiness of vegetal inputs. Some soils were dominated by bulky humic-like compounds (HIX, %C1) that stem from recalcitrant plant matter (e.g. lignin, tannins, humic acids), whereas others were more defined by recently produced low molecular weight compounds (BIX, S_R) (Hansen et al., 2016; Stedmon & Markager, 2005). The latter traits potentially encompassed small root exudates (e.g. organic acids) that plants actively produced to help solubilize nutrients that are commonly limiting in weathered tropical soils (Fujii et al., 2021). Fluorescence from plant phenols (%C4) was unfortunately not detected in our soil samples, possibly due to a lack of accumulation resulting from year-round microbial degradation and/or an overlap with the more prominent protein-like peak (Maie et al., 2007; Zhu et al., 2019). Regardless, it was clear that carcass inputs shifted DOM quality away from plant-centric characteristics. By the end of our study, most CDI soils remained defined by microbially processed DOM compounds, but longer prospective studies are needed to capture the full duration of this disturbance.

The concentrated influx of semi-labile DOM strongly correlated with the emergence of three dominant bacterial classes: Gammaproteobacteria, Bacilli, and Clostridia. These taxa rose in abundance in CDI soils throughout the Active to Dry phases of decomposition, with the most significant peak seen during the Advanced phase. Consequently, native microbes greatly diminished, thus creating a divergence in community richness and evenness. There are two most probable explanations for the close association between DOM chemistry and community

structure. First, the purging of decomposition fluids simultaneously introduced high quantities of semi-labile DOM and pig-associated bacteria. The rise in Clostridia could be attributed to this route, for they were barely present in undisturbed soils. These enteric anaerobes are known to proliferate and translocate within the body cavity following death (Mesli et al., 2017). The phenomenon is so pervasive that Javan et al. (2017) coined the term "Postmortem Clostridium Effect". Clostridia, along with other gut anaerobes like Bacilli, would have been flushed into the surrounding soil following carcass rupture. Herein, they outcompeted resident aerobic groups, as they were able to thrive during periods of hypoxia that formed from excess respiration and/or the saturation of soil pores by decomposition fluids (DeBruyn et al., 2024; Taylor et al., 2024).

Secondly, DOM inputs could have also favoured the growth of certain pre-existing groups. Increasing DOM bioavailability likely alleviated energy limitations for naturally occurring copiotrophs that were otherwise suppressed in the low-resource environment of the tropics (Strickland & Wickings, 2015). This could be particularly true for the r-strategists like Gammaproteobacteria, who have been similarly linked to labile DOM additions in other aquatic and terrestrial systems (Li et al., 2012; Wakelin et al., 2008; Yang et al., 2023). The boom in these three taxa, and the coalescence of enteric and environmental copiotrophs, mirrored several other CDI studies conducted across various ecosystems, soil types and vertebrates (Adserias-Garriga et al., 2017; Cobaugh et al., 2015; Finley et al., 2016; Iancu et al., 2015; Keenan et al., 2023; Mason et al., 2022; Singh et al., 2017; Taylor et al., 2024). Our findings however partly differed from the literature as we did not observe an increase in another constituent of mammalian guts, Bacteroides (Mason et al., 2023; Metcalf et al., 2013). This could be due to contrasting host microbiomes, sequencing-depths and data processing methodologies (e.g. filtering parameters). Interestingly, Mason et al. (2022) did report large variabilities in soil Bacteroides abundances between decomposing human donors. Nevertheless, our findings contribute to the growing evidence of a universal convergence in CDI microbial communities and highlights the importance of DOM composition in structuring these trends.

By the Dry phase of decomposition, the CDI bacterial community still differed from that of undisturbed areas. However, the trajectory of the community's development was starting to reorient itself back towards its starting composition. This was mainly due to a decrease in the

relative abundance of Bacilli and an increase in native groups, specifically Actinobacteria. The bust in Bacilli can be ascribed to the gradual restoration of soil O_2 levels and the observed depletion in DOM quantities (DeBruyn et al., 2024). Improvements in soil conditions and reduced heterospecific competition would have then allowed Actinobacteria to occupy a newly available niche. This taxa's ability to degrade complex forms of DOM (Rebai et al., 2024), as supported in this study by its positive correlation with humic characteristics (HIX, %C1), would have enabled it to rapidly utilize bulky aromatic compounds that arose from the earlier processing of carcass materials. The comeback of Actinobacteria was equally observed by Cobaugh et al. (2015) in Tennessee soils beneath decomposing human remains. While it appeared that the community was trending towards baseline, a significantly longer amount of time would have been needed to capture a full recovery. Burcham et al. (2021) reported disruptions in beta diversity metrics in pig CDIs nearly a decade after carcass placement and Singh et al. (2017) still detected a divergence in bacterial communities of human CDIs up to 2 years post-deposition. Identifying and understanding how specific factors like DOM composition interact with soil microbial communities can guide the development of more targeted strategies to help accelerate the remediation of carrion-related disturbances.

DOM-driven changes in bacterial community structure were moreover linked to an observed shift in metabolic activity and function. Absolute bacterial abundance (BA) rapidly peaked underneath the carcasses (0 cm) during the Advanced and Dry phases of decomposition. Our flow cytometry results showed an increase in BA, which conflicted with previous qPCR-based studies that reported little to no change (Cobaugh et al., 2015; Taylor et al., 2024). This does not necessarily reflect discrepancies between findings, but rather differences in the techniques' target. The use of flow cytometry enabled us to enumerate intact cells (dead and alive), whereas qPCR quantifies gene copies, which can vary depending on the bacterial species, growth phase and the presence of extracellular DNA (Galazzo et al., 2020; Klappenbach et al., 2000; Ludwig & Schleifer, 2000). The elevated cell numbers in our soils were most correlated with the relative abundance of Clostridia and Gammaproteobacteria. While Bacilli occupied the greatest relative abundance, Clostridia and Gammaproteobacteria appeared to be more dominant in terms of total cell numbers, thus hinting at an increase in growth and survival of taxon members (Lin & Peddada,

2020). These two groups were also related to the degradation of labile carboxylic acid substrates, such as itaconic acid (CA5), a carbon compound commonly produced by mammalian immune cells as an endogenous antimicrobial (Cordes et al., 2015). Heo et al. (2020) previously identified this substrate as a potential indicator of vertebrate decomposition in one of two pig trials. We also saw the preferential metabolism of the neurotransmitter Gamma-amino butyric acid (CA3), which is involved in the gut-brain axis and is extensively produced by enteric and bacterial cells (Belelli et al., 2025). D-Glucosaminic acid (CA4) and D-Galacturonic acid (CA2) were likewise metabolized and can be respectively connected to the oxidation of the connective tissue component glucosamine (Chen et al., 2010; Wu et al., 2011) and the intestinal fermentation of plant pectin (Yüksel et al., 2024). The shared utilization of carboxylic acids by Clostridia and Gammaproteobacteria reflects functional redundancy and points to their collective role in degrading compounds originating from carcass tissues and damaged microbial decomposers.

We also observed elevated rates of bacterial respiration (BR) during the Active and Dry phases. This effect extended to -20, 0 and 20 cm sample sites and was not proportional to changes seen in BA, therefore indicating a veritable increase in cell-specific respiration. Heightened BR is a widespread observation in CDI soils and reflects the intense energy demands of the soil community (Burcham & Jordan, 2017; Carter et al., 2007; DeBruyn et al., 2024; Mason et al., 2023). The enhanced use of carbon could originate from the emergence of highly active copiotrophs that are exploiting labile inputs. Alternatively, it may reflect environmental stress caused by changes in CDI conditions (e.g., pH, nutrient loads). Under such stress, bacteria will divert carbon toward metabolic maintenance rather than biomass production, as shown by the reduction in bacterial growth efficiencies in two separate human CDI studies (Cobaugh, 2013; see Chapter 3). We found that this increase in activity was positively correlated with the relative abundance of Bacilli. It may seem counterintuitive that an enteric group is responsible for the highest rates of aerobic respiration, especially at a point in decomposition when O_2 levels are assumed to be low (Taylor et al., 2024). However, many Bacilli are facultative anaerobes that can switch metabolic strategies as soils transition from oxic to hypoxic conditions (Miguel et al., 2023). Even under hypoxia, Bacilli could consume residual O_2 still contained within the diffusion gradient or in soil pores. Aeration from sample preparation undoubtedly favoured aerobic respiration,

which is not entirely representative of the CDI environment. Despite these biases, the results demonstrate how the combination of carcass inputs and bacterial community composition is conducive to high BR in the presence of oxygen. Bacilli and BR were moreover associated with the degradation of several labile carbohydrates (C2, C4, C5, C6), most of which are likely gut fermentation products (Stülke & Hillen, 2000; Yoon et al., 2003). The extensive repertoire of carbohydrate-specific enzymes and transporters that Bacilli are known to have would enable them to effectively utilize sugars as a primary energy source (Manzo et al., 2011; Stülke & Hillen, 2000). These findings underscore the potential role of Bacilli in leading respiration and carbohydrate metabolism in CDI soils. Its separation from Clostridia and Gammaproteobacteria implies niche portioning within the CDI community, which could facilitate the turnover of a broader range of carcass-derived materials.

Comparisons between the tropical Hawaiian site and a temperate Quebec site showed variable bacterial responses despite equivalent changes in DOM composition. Even with a difference in carcass starting masses (40 kg vs 90 kg), a comparable relative increase in DOC was produced at the 0 cm mark. An analogous change in DOM quality was also seen with the influx of carcass materials. In both cases, carcass decomposition improved DOM lability by shifting the composition from vegetal humic-like to more microbial protein- and fulvic-like characteristics. These similar patterns in DOM composition across biogeographic regions stresses the conserved chemical makeup of carcass tissues and the universal importance of microbially-driven decomposition processes. Bacteria in Hawaiian soils however appeared more sensitive to carcass inputs, generating a larger relative increase in cell numbers and respiration rates when compared to Quebec. The BioLog EcoPlate™ profiles of the temperate community were also principally dominated by carbohydrate metabolism, which contrasts with the mixed carbohydrate and carboxylic acid profiles of Hawaiian CDIs. Body decomposition has previously been shown to direct a convergence in CDI microbial communities, regardless of regionality (Burcham et al., 2024). If we assumed that the bacterial communities were similar across Hawaiian and Québec CDIs, then the difference in responses can be only ascribed to environmental factors. Ambient temperature can be excluded, as daily averages across both sites hovered around 22-23°C at the time of sampling. Edaphic properties however greatly differed. The near neutral, well-drained

and granular-blocky structure of the Hawaiian site's soil (Pamoa series) may have favoured enhanced responses by supporting aeration and enzymatic activity (USDA, 2001). This is in contrast to the acidic and gleyed soil (Achigan series) of the Québec site (Soil Landscapes of Canada Working Group, 2010). Additional differences in the interactions between CDI and native taxa, or substrate adsorption between clayey and sandy soils, could have moreover contributed to the observed dissimilarities in functional profiles. By comparing the two studies, we were ultimately able to reveal inter-site variations in CDI microbial responses. To our knowledge, this sort of biogeographical comparison is the first of its kind and demonstrates a need to further identify regional modulators of SOM-bacterial dynamics in CDIs. These preliminary findings underline the sensitivity of Hawaiian soils and the need for a curated approach to managing carrion in this region.

Conclusion

This study established important relationships between the chemical characteristics of SOM and soil microbiology in the context of animal decomposition. We demonstrated that carrion provides a significant input of semi-labile compounds to soil systems, which supports the development of a distinct bacterial community that is dominated by intestinal anaerobes and high-energy copiotrophs. Furthermore, we revealed functional differences among bacterial taxa that collectively resulted in the preferential respiration and degradation of labile carcass materials. While these functional shifts facilitated the processing of carcass materials, if persistent, they may overlook the turnover of recalcitrant plant compounds that still exist in and around CDIs.

Additionally, our study offered novel biogeographic comparisons that highlighted the sensitivity of Hawaiian soil microbiota to carrion-derived SOM inputs. We acknowledge that our dataset is limited in both its duration (20 days) and its inclusion of historical (e.g., land use), environmental, and edaphic (e.g. soil texture, O_2 levels) data. Such information would help to further define the duration of carrion-related disruptions and to identify regional regulators of bacterial responses. Nonetheless, the insights gained from this research will enhance our understanding of the role of carrion plays as both a disturbance and a resource in tropical

ecosystems. Models of energy and nutrient budgets, as well as carrion management strategies, will benefit from these findings, especially as global animal mortalities continue to increase (Barton et al., 2019).

Acknowledgements

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Supplementary material

Text S4.1 – Detailed methodology for PCR amplification.

2 µL of DNA extract was mixed with 1 µM of forward and reverse 16s RNA primers (515/806R) and 12.5 µL of OneTaq® DNA polymerase master mix (2X in standard reaction buffer, New England BioLabs Inc., Ipswich, MA). The reaction was then completed to a final volume of 25 µL with nuclease-free water. PCR amplification was performed by running an initial denaturation cycle at 94°C for 30 seconds, followed by 35 cycles of: denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 68°C for 60 seconds. A final extension was performed for 5 minutes at 68°C. DNA amplification was confirmed by electrophoresis on a 1% agarose gel. PCR products were kept at -20°C prior to being sent for sequencing.

Text S4.2 – Details of bioinformatics workflow using DADA2.

Adaptors and primers were first trimmed from the sequences using the *Cutadapt* (Martin, 2011) tool for Linux. Subsequent steps were then performed using functions from the DADA2 R package (Callahan et al., 2016). Low quality reads (Q-score < 30) were truncated from sequences using standard parameters truncLen=c(260, 245), maxN=0, maxEE=c(2,2) and truncQ=2. Sequencing error rates in forward and reverse reads were estimated using the *learnError* function. From this, sample composition was inferred and denoised ASVs were generated according to the DADA algorithm. Forward and reverse reads were aligned and merged with an overlap of at least 12 nucleotides and a mismatch threshold of zero using *mergePairs*. Chimera sequences were detected and removed by applying the consensus method of the *removeBimeraDenovo* function. Finally, taxa were assigned with a 99% sequence identity threshold using *assignTaxonomy* and the SILVA ribosomal RNA gene database (Quast et al., 2013).

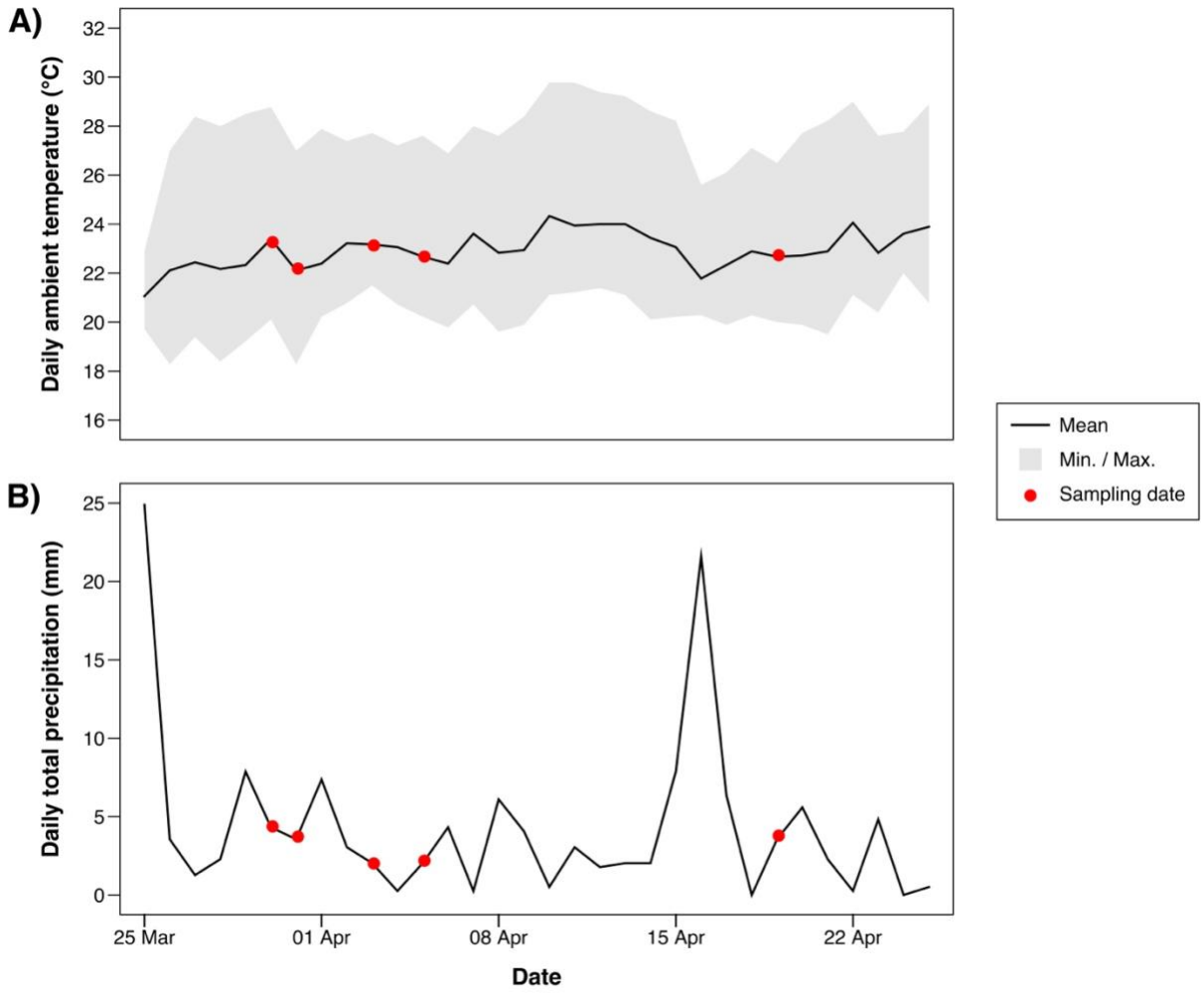

Figure S4.1 – Local meteorological data throughout the duration of the Hawaiian pig decomposition study (March to April 2022).

Mean daily ambient temperature (**A**) and total precipitation (**B**). Soil sampling dates are indicated by a red dot (•). Publicly available historical data was retrieved online from a nearby weather station (Weather Underground, 2024) located in the Palolo Valley, HI (21.303° N, 157.794° W).

Figure S4.2 – Soil sampling design for the Hawaiian decomposition study.

Soil cores (circles) were taken at incremental distances uphill (-50, -20 cm), underneath (0 cm) and downhill (20, 50 cm) from surface deposited pig carcasses (H1, H2, H3). Additional downhill cores (squares) were taken at a distance of 100 cm and 200 for pigs H2 and H3. These distances were not sampled for H1 due to a cliffside and thick vegetation.

Figure S4.3 – Visual state of pig carcass H3 during each decomposition phase and sampling event.

A) Fresh (day 0), **B**) Bloat (day 1), **C**) Active (day 4), **D**) Advanced (day 6), **E**) Dry remains (day 20). Carcasses were deposited 30 March 2022.

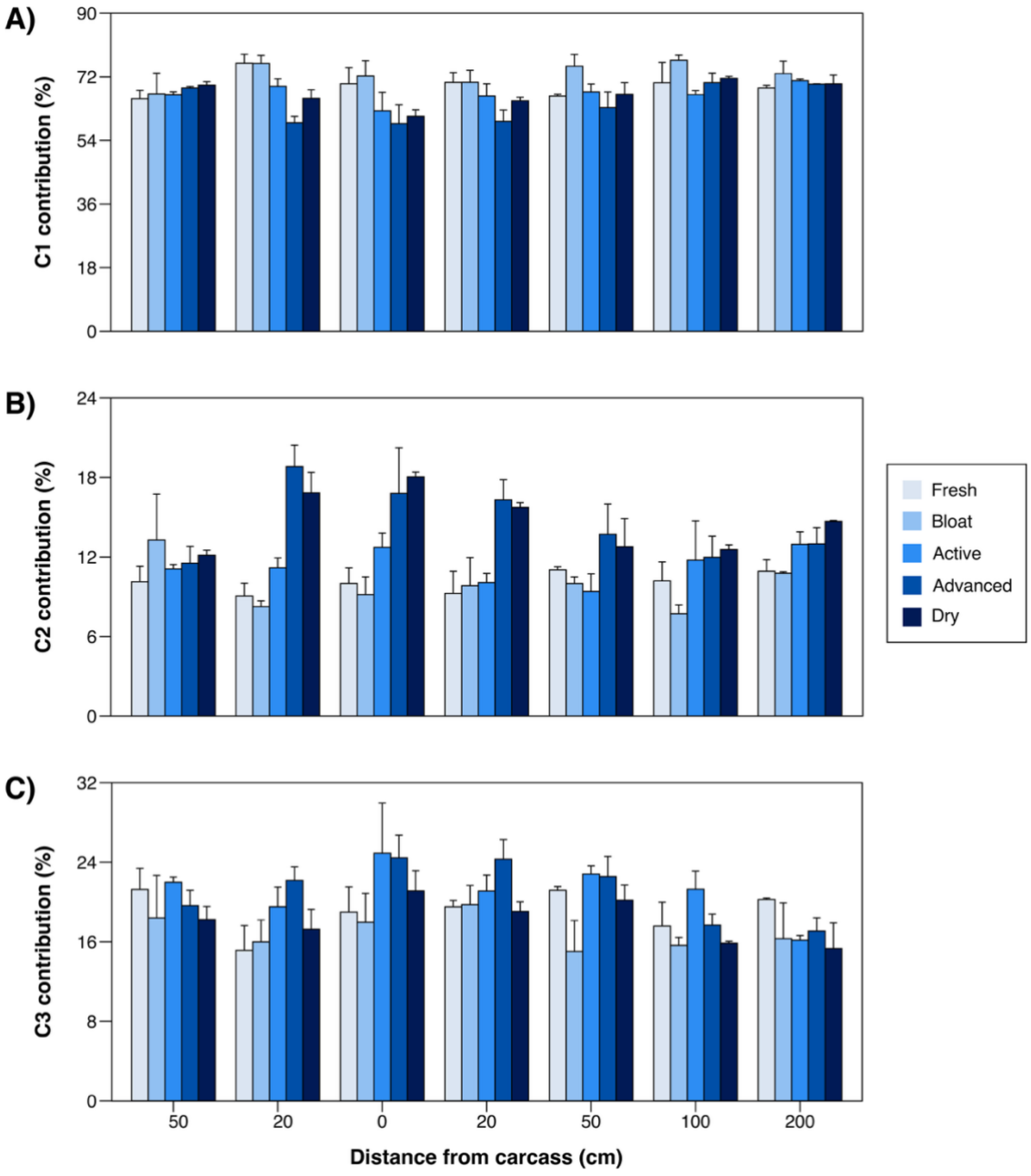

Figure S4.4 – Relative percent contribution of PARAFAC components in soils sampled around decomposing pig carcasses in Hawaiian tropical savanna ecosystem.

Mean and standard error of components C1 (**A**), C2 (**B**) and C3 (**C**) obtained from soil collected underneath (0 cm) and at varying distances uphill (-50, -20 cm) and downhill (20, 50, 100, 200 cm) from pig carcasses during each phase of decomposition (Fresh, Bloat, Active, Advanced, Dry).

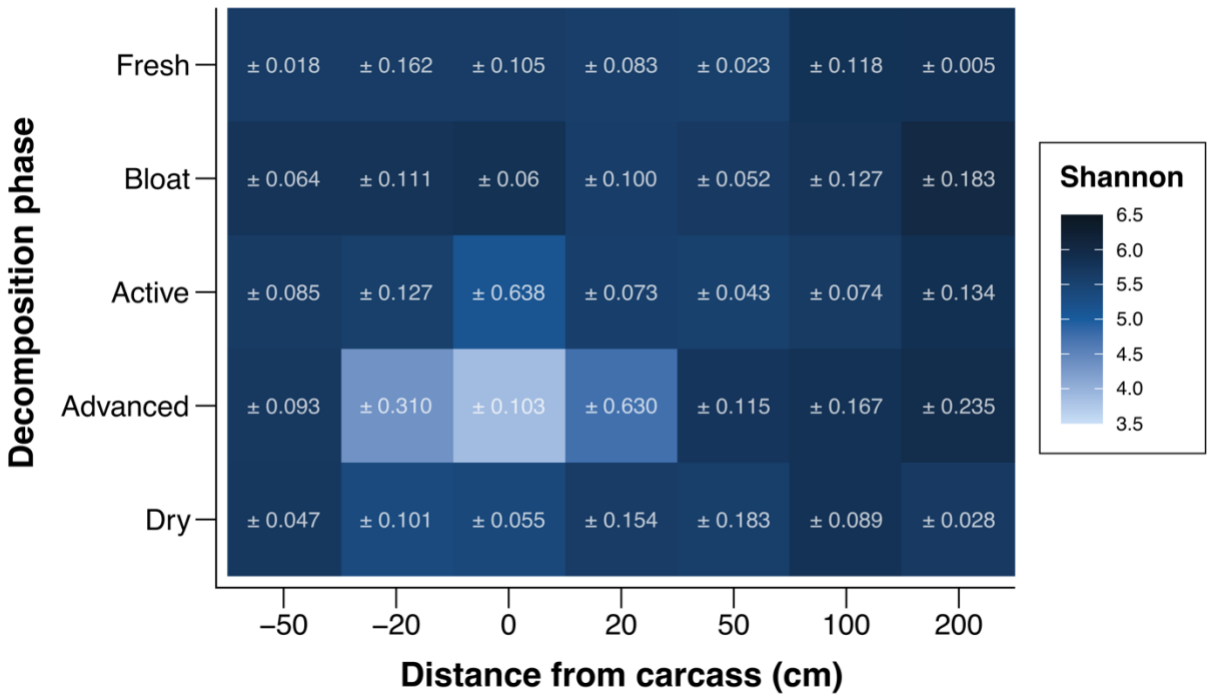

Figure S4.5 – Alpha diversity of soil bacterial communities around decomposing pig carcasses within a Hawaiian tropical savanna ecosystem.

Mean ± standard error of the Shannon diversity index calculated from the relative abundance of bacterial ASVs.

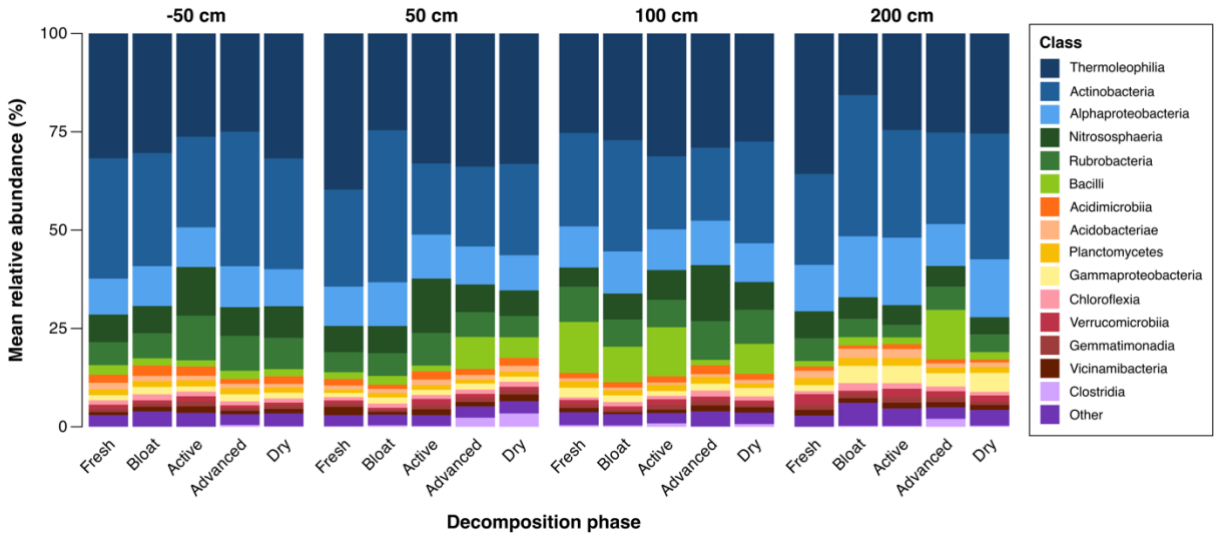

Figure S4.6 – Relative abundance of bacterial taxa, at the class level, in soils that were least affected by pig carcass decomposition within a Hawaiian tropical savanna ecosystem.

Top 15 most abundant classes are displayed. Remaining classes are grouped as “Other”.

Table S4.1 – Primers sequences used for PCR amplification of bacterial DNA

Target	Primer	Sequence
16S rRNA (V4)	515F	5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGYCAGCMGCCGCGGTAA-3’
	806R	5’- GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTACNVGGGTWTCTAAT-3’

Table S4.2 – PCA loading values for variables of DOM composition.

Variable	PC1	PC2
DOC	0.483	0.078
%C1	-0.295	-0.461
%C2	0.437	0.180
%C3	0.104	0.532
FI	0.461	-0.043
HIX	0.205	-0.498
BIX	-0.270	0.428
SR	-0.388	0.180

Table S4.3 – PCA loading values for Biolog EcoPlate™ substrates.

Variable	PC1	PC2
A1	-0.267	-0.050
A2	-0.198	-0.042
AA1	0.186	-0.148
AA2	0.157	-0.325
AA3	-0.118	-0.177
AA4	-0.177	-0.127
AA5	0.066	-0.163
AA6	-0.062	0.131
C1	-0.122	-0.148
C2	0.239	-0.111
C3	-0.065	-0.249
C4	0.174	0.018
C5	0.249	-0.041
C6	0.040	-0.155
C7	0.064	-0.074
C8	-0.143	-0.348
C9	-0.050	-0.071
C10	-0.047	-0.230
CA1	-0.197	-0.194
CA2	0.256	-0.081
CA3	0.236	-0.173
CA4	0.196	-0.192
CA5	0.239	-0.134
CA6	-0.060	-0.261
CA7	-0.268	-0.006
P1	-0.257	0.022
P2	-0.276	0.015
P3	-0.123	-0.270
P4	-0.120	-0.301
PC1	-0.156	-0.258
PC2	0.224	-0.206

Table S4.4 – Variable of importance to Projection (VIP) scores from a PLSR of DOM vs bacterial taxa.

Variables that are most important are identified by a score greater than 1 (in **bold**).

Variable	Comp 1	Comp 2
DOC	**1.51**	**1.46**
C1	**1.13**	**1.12**
C2	**1.32**	**1.27**
C3	0.640	0.757
FI	**1.08**	**1.05**
HIX	0.301	0.515
BIX	0.503	0.569
Sr	0.886	0.873

Table S4.5 – Variable of importance to Projection (VIP) scores from a PLSR of bacterial taxa vs bacterial activity/function.

Variables that are most important are identified by a score greater than 1 (in **bold**).

Variable	Comp 1	Comp 2
Bacilli	**1.11**	0.998
Actinobacteria	0.785	**1.08**
Thermoleophilia	1.00	0.925
Nitrososphaeria	**1.04**	0.956
Alphaproteobacteria	**1.08**	**1.19**
Gammaproteobacteria	0.937	0.967
Rubrobacteria	0.990	0.947
Clostridia	**1.02**	0.905

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Chapter 5 – Conclusion

Vertebrate decomposition acts as both a resource and disturbance within terrestrial ecosystems, primarily through the leaching of organic matter into surrounding soils. While research on plant litter has shown that interactions between SOM chemistry and bacterial communities are central to the cycling of organic inputs, these processes remain poorly characterized in vertebrate CDIs. In consequence, the fate of vertebrate-derived inputs and their broader environmental impacts are not well understood. This dissertation addressed these gaps by evaluating the spatiotemporal effects of human and pig decomposition on SOM–bacterial dynamics. Results showed that the SOM pool in CDIs underwent distinct shifts in quantity, source, and lability, which in turn stimulated its turnover through the reshaping of bacterial activity levels, metabolic function, and community structure. Cold seasonal conditions were found to influence the magnitude and onset of CDI changes (Chapter III), but did not cease the transformation of inputs (Chapter II). Relative shifts in SOM chemistry were moreover comparable across geographic regions but elicited dissimilar bacterial responses (Chapter IV). The information collected from across biogeoclimatic conditions will provide a more mechanistic understanding of vertebrate's role in biogeochemical cycles and the maintenance of ecological processes, something that is of interests in both ecology and forensic taphonomic research.

DOM-bacterial dynamics

Decomposition-related changes in the SOM pool (points 1 and 2 of **Figure 1.2**) were successfully identified throughout this doctoral project by analyzing a combination of DOC concentration, DOM optical properties and $^{13}C/^{15}N$ stable isotopes. Vertebrate decomposition was found to create a large influx in organic matter compounds with characteristics that are indicative of a microbial (Chapters II-IV) and vertebrate (Chapter III) source. These inputs produced a drastic shift in molecular traits, moving the DOM fraction from predominantly aromatic vegetal humic-like compounds to smaller, more aliphatic microbial fulvic-like and/or protein-like compounds (Chapters II-IV). This was conducive to a major increase in both quantity and quality.

Building on these chemical changes, Chapter IV demonstrated a close link between vertebrate SOM inputs and bacterial succession. Decomposition fluids carried enteric bacteria (Clostridia, Bacilli) and labile compounds that further supported the emergence of copiotrophic groups (Gammaproteobacteria). The selection towards these CDI groups were mirrored by a decrease in the relative abundance of native groups, thus resulting in an overall diminishment in α- and β-diversity metrics. SOM chemistry and its associated community changes were moreover seen to trigger strong bacterial metabolic responses (Chapters II and IV). These responses, which were observed across all studies, consisted of a heightened cell-specific respiration and greater metabolic potential for reducing vertebrate-associated labile carbohydrate and carboxylic acid substrates. This ultimately revealed how vertebrate decomposition selects for the bacterial degradation of its own SOM inputs (points 3 and 4 of **Figure 1.2**).

The increased bacterial propensity for vertebrate SOM ensured a rapid depletion of inputs, as seen through decreasing DOC levels. BGE values in Chapter III further helped to distinguish where those inputs were going, as matter can only be transformed, not destroyed. Soil bacterial biomass production was substantially reduced relative to respiration rates, meaning that a greater portion of carbon, and by proxy SOM, was being shuttled towards volatilization rather than immobilization. Enrichment in ^{15}N additionally corroborated the occurrence of enhanced mineralization and gaseous loss. This sheds light on which pathways should be emphasized when studying and modelling the role of vertebrate decomposition in biogeochemical cycles.

A second shift in SOM chemical characteristics was moreover observed near the end of vertebrate decomposition (Chapters II and III). The rapid turnover of labile vertebrate inputs seemed to humify residual DOM, resulting in an increase in aromaticity and molecular complexity (point 4 of **Figure 1.2**). These compounds could have arisen from a combination of microbial biomass-components, selective preservation and/or spontaneous polymerization. Interestingly, the DOM at this stage shared optical properties typically associated with both microbial and vegetal sources. It sat as an intermediate between labile inputs and the humic vegetal signal of undisturbed soils. Transformations of vertebrate inputs could have resulted in the evolution of

distinct compounds that have not yet been optically characterized. The persistence of these compounds however still remains unknown.

The research done as part of this doctorate successfully identified key patterns and interactions between SOM chemistry and bacterial responses within CDIs. This has revealed how vertebrate decomposition inputs are utilized and cycled while simultaneously acting as a bacterial selective pressure. Through these SOM-bacterial dynamics, we now have a more mechanistic understanding of the dual role CDIs play as both a resource and disturbance within terrestrial environments.

Spatiotemporal patterns

All three studies (Chapters II-IV) demonstrated consistent spatial patterns for the peak onset of SOM and bacterial changes. Disturbances across all measures, sites, and seasonal conditions were laterally constrained to a sampling radius of 20 cm, with little to no changes detected at 50 cm. Variations could have occurred between the two sampling points (e.g., 30 and 40 cm), but this was not captured with the current sampling design. Decomposition-related shifts in SOM and bacterial measures were largely restricted to the top mineral layer of soil (A-horizon), with only a faint increase in DOC being observed in the deeper B-horizon of human CDIs (Chapter III). By analyzing samples according to horizons rather than absolute depth, the reported CDI patterns are interpretable within the context of a functional and structural unit of soil, which can be easily identified and parsed out across different sites. Variations in erosion, litter accumulation and pedology can complicate cross-site comparisons when using absolute depths, hence the use of horizons aided with standardization. Through this doctoral project, it was nevertheless found that moderately sized vertebrates (~40-90 kg) have a highly constrained spatial effect on SOM-bacterial dynamics. This information is valuable for delimiting areas that may warrant further study, monitoring or mitigation following vertebrate decomposition.

Temporal patterns however varied across absolute timescales between studies and conditions. Peak SOM-bacterial changes in Hawaiian pig CDIs were observed around days 6-20 (Chapter IV), whereas they appeared between days 227-364 for cold-deposited human donors at REST[ES] (Chapter III). These timelines may seem drastically different, but they do share

something in common when examined from a taphonomic perspective. The onset of CDI changes regularly occurred during the Advanced phase of decomposition. At this point, soils had become fully saturated with fluids that had been purged since the start of the Active phase. The introduction of these fluids thereby marked the point in which CDI changes were elicited. Most CDI disturbances eventually exhibited a trend towards baseline by the Dry phase of decomposition. The pig study of Chapter II especially showed a near recovery of DOC levels and bacterial metabolic responses by the subsequent spring season, 324 days after deposition. Yet, SOM chemical characteristics in CDIs remained strongly distinct till the end of each study, mainly driven by an enrichment in microbial compounds and stable isotopes (^{13}C, ^{15}N). The full temporal extent of these chemical changes unfortunately couldn't be captured within either study's timeframe, for they are likely to persist for a considerable amount of time. Longer prospective studies are therefore needed to determine their exact duration and legacy effects. The Advanced phase nevertheless appears to be practical benchmark that can be adopted by decomposition site managers, HTFs and animal mortalities alike, to initiate environmental monitoring and remediation efforts. Overall, the findings from this doctoral project revealed temporal differences in bacterial and SOM disturbances but, more importantly, highlighted the value of integrating decomposition milestones when assessing soil-based changes in CDIs.

Seasonal and regional comparisons

This dissertation is novel in its examination of SOM-bacterial dynamics in CDIs, but it is also unique in its emphasis on temperate seasonal conditions (point 6 in **Figure 1.2**). The vast majority of vertebrate decomposition research has taken place at lower latitudes where seasonal variations are relatively mild. Contrarily, the northern temperate region of Québec, Canada experiences climatic extremes throughout the year . Chapter II specifically examined how SOM and bacterial measures in CDIs evolved over subsequent seasons. It was with great surprise that SOM transformations did not cease over the colder months. Between the autumn and spring, pig CDIs experienced a depletion in DOC levels and a greater humification of DOM compounds. Logistical difficulties of accessing the carcasses and CDIs under deep snow meant that no soil was sampled during the winter. It is therefore difficult to determine if these transformations were due to microbial activity or physicochemical processes created by cold temperatures, freeze-thaw

cycles and/or snowmelt. Given existing findings on cold-tolerant bacteria, it is rational to believe that some of transformations were the product of sustained microbial activity. Chapter III additionally had a climatic angle by evaluating SOM-bacterial dynamics according to donor deposition season. The sequence of CDI chemical and bacterial changes were the same between all donors, but donors who experienced cold conditions produced changes at a lower magnitude and later absolute timepoint. Tissue preservation from overwintering delayed the onset of the Advanced phase and reduced the availability of labile materials. Regardless, cold donors still had a noticeable effect on SOM-bacterial dynamics, which was considerably prolonged in comparison to their warm counterparts. Together, these two studies demonstrated that vertebrate decomposition still has an important impact on soil biogeochemistry even in the face of cold seasonal conditions. The influence of cold seasons should therefore not be overlooked in CDI research, nor in the development of environmental management strategies.

Data on regional variability is greatly needed in decomposition research, yet it is seldomly collected. Chapter IV addresses this gap through its comparison of Québec (temperate) and Hawaiian (tropical) CDI biogeochemistry (point 6 in **Figure 1.2**). Pig carcasses across study sites produced comparable relative changes in SOM composition, reflecting a degree of universality in the chemical makeup of vertebrate bodies and their decomposition fluids. In contrast, bacterial metabolic responses diverged. Tropical Hawaiian CDIs exhibited a stronger relative increase in bacterial activity and a greater potential for degrading carboxylic acid substrates. This heightened activity may reflect an increased sensitivity to vertebrate inputs, while the distinct substrate preferences point to region-specific functional outcome. Such differences likely stemmed from how emerging CDI bacterial communities interact with regionally specific edaphic features, climate, native taxa and baseline resource levels. Further investigations will be needed to identify geographical drivers of these bacterial dissimilarities. The findings of Chapter IV conclude that decomposition chemistry may be globally predictable, but its microbiology is distinctly regional. This builds the argument that CDI treatments will need to be locally adapted.

Limitations and future directions

The findings presented offer valuable insights, but they must be interpreted within the context of certain limitations, many of which have already been noted throughout this dissertation. The most substantial constraint is the limited statistical power resulting from small sample sizes. This was especially evident in Chapters II and IV, where the use of only three pigs made it difficult to obtain statistical significance despite clear trends, data transformations, and mixed-effects modelling. Sample sized in decomposition research are commonly hindered by the high cost and scarcity of carcasses and/or human donors, project time restrains and by space limitations at HTFs or animal research sites. A sample size of n = 3 was adopted throughout this doctorate as it gave the minimum degrees of freedom required to capture nature variability (i.e. variance, standard deviation) while still respecting logistical constraints (Quinn et al., 2002). In light of this limitation, the studies presented here placed greater emphasis on descriptive results rather than statistical outcomes. While this may hinder the generalizability of the findings, it does not diminish their value. The clustering of individuals and CDI samples, in addition to the consistency in spatiotemporal trends across analysis types (chemical, bacterial) and their biological plausibility, is evidence in itself of the strong effect that vertebrate decomposition has on soil biogeochemistry. The findings from this dissertation will provide a valuable basis for refining future hypotheses and experimental designs, thus setting the stage for more statistically sound studies. With each new study, similarities in findings will help offset these statistical shortcomings by mutually validating the observed phenomena and gradually building the empirical foundation needed for the development of robust concepts

Another limitation that must be acknowledged is the removal and exclusion of the top O-horizon. This organic-rich layer was discarded due to its temporal and spatial variability within an experimental site. Seasonal changes in leaf-litter, physical disruptions from footsteps and the accumulation of sticky decomposition residues resulted in the irregular presence of the O-horizon across soil cores. The layer was therefore removed to help improve sample standardization. It must however be recognized that the O-horizon, when undisrupted, is an important site of microbial biomass, soil formation and organic matter turnover (Coleman et al., 2018). Processes within this layer would undoubtedly contribute and regulate the environmental impact of

vertebrate decomposition. Due to its direct contact with the body and ambient conditions, the O-horizon would likely be more susceptible to decomposition-related changes and have a greater seasonal and/or regional variability. When compared to the mineral A-horizon, the O-horizon's greater bacterial load would likely amount to greater respiration rates and faster transformation of SOM. The intensity of microbial processes may also mean that CDI effects are more transient in the O-horizon, whereas the lower mineral horizons will serve as a site for the prolonged accumulation and retention (i.e. adsorption) of recalcitrant compounds (Schulze et al., 2009; Torn et al., 2005). The latter of which would be more suitable for understanding long-term impacts or for the developing forensic markers of late-stage decomposition. It must however be kept in mind that the O-horizon likely plays an important role in the overall SOM-bacterial dynamics of CDIs.

It must also be noted that the biogeographical comparisons made in Chapter IV (Hawaii vs Québec) did present some confounding variables between studies, most notably differences in carcass mass. Québec carcasses had a starting mass of ~90 kg, whereas the Hawaiian pigs were much smaller at ~40 kg. Although this difference could have influenced taphonomic processes and the quantity of soil inputs, that effect was likely minimal on the examined biochemical measures. First, both Hawaii and Québec CDIs presented similar increases in relative DOC levels, thus implying that a comparable level of organic matter was present at the time of sampling. If body mass was an influencing factor, the Québec pigs would have moreover produced the greatest increase in CDI bacterial responses from the larger influx of carcass materials and enteric microbes. This was however not the case, thus implying that more poignant factors were responsible for the heightened responses observed in the Hawaiian CDIs. This reveals an important need to examine additional factors and their potential role in inter-site differences, as well as response outcomes (e.g. to explain low BGE). This could include, but is not limited to, edaphic properties, weather and local microbial communities. Integrating such factors into future multivariate analyses can help us identify key modulators of vertebrate decomposition and its environmental impact.

Beyond identifying additional factors that shape CDI biochemistry, future work will also need to address vertebrate decomposition at a broader depositional, temporal and ecological scales. HTFs and mass-mortality events often involve multiple remains that are spatially and

temporally clustered. We will need to determine whether the amassment of multiple bodies, or deposition onto pre-existing CDIs, produces additive or synergistic effects on soil chemistry and microbiology, while also accounting for other key microorganisms such as fungi, protists, and archaea. Longer prospective studies (> 1 year) will next be necessary to determine "*if"* and *"when"* CDI soils become naturally remediated, particularly for persistent chemical disruptions like stable isotope ratios and SOM quality. Point 5 of this dissertation's conceptual framework (**Figure 1.2**) will equally require deeper investigation, so that we can better tie in CDI biogeochemical changes to downstream ecological outcomes. This can be through including measures of plant nutrient uptake, primary productivity and biodiversity. Ultimately, these directions will help bridge the gap between localized decomposition effects and their broader environmental significance.

Despite its limitations and need for future development, this doctoral project yielded important insights into the effects of vertebrate decomposition on key soil processes, especially bacterially mediated SOM cycling. The findings and conclusions can already inform environmental management and mitigation strategies for sites of vertebrate decomposition. This work's knowledge can be synthesized into the following preliminary recommendations, which will continue to evolve as the field of research progresses.

(i) *Remains can be placed closer together (~ 50 cm)*. Surface-deposited remains may be placed in closer proximity) without there being a substantial risk of cross-contamination between soil bacterial communities and SOM compounds.

(ii) *Remove remedial remains and residues, then replenish the soil*. Tissues and fatty deposits should eventually be removed, as this may have an extended effect on SOM chemistry and soil bacteria, even if decomposition has appeared to have visibly ended (dry remains /skeletonization, preservation). Afterwards, undisturbed soil and leaf litter that was locally collected should be placed over the CDI to help re-introduce native bacterial groups and vegetal-associated SOM compounds. The CDI should be cordoned off (> 1-year) so that the newly forming litter and soil is not disturbed.

(iii) *Initiate environmental strategies at the onset of the Advance phase*. Peak soil disruptions commonly appear following the Advance phase of decomposition. It is therefore a suitable benchmark to commence monitoring programs or remediation timelines.

(iv) *Cold seasons should not be disregarded*. Decomposition and microbial processes can persist under cooler climatic conditions. Therefore, monitoring and management should not be halted or ignored during cooler or snowy periods.

(v) *Monitoring and site-specific adjustments will be needed*. Due to geographical differences in bacterial responses, sites should monitor their soils in the early days of operation in order to make site-specific adjustments to their practices.

The above recommendations establish a foundation for evidence-based management of vertebrate decomposition sites. These will continue to evolve as new developments are made within both the fields of ecology and forensic taphonomy. This dissertation provides a stepping stone toward a more sustainable management of vertebrate decomposition in terrestrial ecosystems. This will not only benefit the health of local environments but also contribute to improving the quality of taphonomic research and soil-based forensic techniques.

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