RESEARCH ARTICLE

# Degraded ecosystem soil and type 2 diabetes gut microbiomes share altered potential metabolism for sugars, lignin and branched-chain fatty acids: a blind spot for global health?

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## Abstract

The global rise in human allergic and autoimmune diseases is linked to altered environmental microbiome exposures from increasing land use change, ecosystem degradation and biodiversity loss. Yet, microbiome-mediated impacts of global change on metabolic health have received little attention. Type 2 diabetes (T2D) is a complex metabolic disease with rapidly increasing global prevalence, uncertain pathogenesis and influences from gut microbiome-host interactions and environmental factors. Soil microbiomes shift in composition and functional capacity with ecosystem condition and are important for supplementing the human gut microbiome and contributing to food quality, both of which are critical in metabolism. Here, we sought to examine overlapping functional capacities in model case study microbiome datasets from (1) soils from a gradient of ecosystem quality (from highly disturbed to mature/natural sites under forest ecosystem restoration) and (2) gut microbiomes in T2D versus normal health to investigate plausible links between ecosystem degradation and metabolic anomalies encoded in T2D gut microbiomes. We developed a novel method to translate microbiome functional capacities (i.e., functional gene-associated biochemical pathway relative abundances) to 'compound processing potential' (CPP, %), reflecting the potential metabolism of individual compounds. Examining selected compounds relevant to soil and gut health, and via exhaustive compound-wide trend analyses, we identified compounds that shared potential metabolism trends. We found increased potential metabolism for sugars and decreased potential metabolism for lignin and monomethyl branched-chain fatty acids, in both T2D and degraded ecosystems. These trends were confirmed in separate T2D case study cohorts from Sweden and China, and degraded versus more natural biodiverse soil-plant systems in the United States and Australia, suggesting a generalizable pattern. Our findings support a consequential new hypothesis that excessive exposures to degraded ecosystem soil microbiomes, and/or nutrient-depleted food derived from such systems, may distort gut microbiome functional capacities and metabolism and thereby contribute to type 2 diabetes.

53 # 1. Introduction

54 Environment and lifestyle exposures under the global trends of land use change, ecosystem
55 degradation, biodiversity loss, urbanization and reduced nature contact are associated with many non-
56 communicable diseases, including several with suspected microbiome-mediated links (Flandroy et al.,
57 2018; Flies et al., 2019; Roslund et al., 2022). Among these, type 2 diabetes (T2D) is a major chronic
58 multifaceted metabolic disease with uncertain pathogenesis, characterized by insulin resistance, loss of
59 insulin secretion, high blood sugar levels and wider metabolic dysregulation (Beulens et al., 2022; Lu
60 et al., 2024; Pearson, 2019). Growing evidence supports the influence of gut microbiomes on human
61 metabolic health and dysfunction. However, there is a pressing need to better understand the
62 contribution of microbiomes in T2D (Cunningham et al., 2021; Gurung et al., 2020) due to their
63 potential mediating influence via diet, lifestyle and environmental factors. The role of environmental
64 microbiomes in supporting human metabolic health remains understudied (Flandroy et al., 2018)—
65 particularly in the context of accelerating global change (IPBES, 2018). T2D is a major contributor to
66 the global non-communicable disease pandemic (Cheema et al., 2014) and represents ~96% of total
67 diabetes cases, which impacted 529 million people in 2021 (6.1% prevalence; costing US$966 billion),
68 and is expected to rise to 1.31 billion people by 2045-2050 (10% prevalence; costing US$1.05 trillion)
69 (Ong et al., 2023; Sun et al., 2022).

70 The human gut microbiome is directly involved in our metabolism, shaping the biochemical
71 profile of our diet via an extensive metabolic repertoire that is distinct yet complementary to
72 mammalian digestive enzymes (Rowland et al., 2018). The gut microbiome and its diet-derived
73 metabolites influence human physiology and energy regulation by modulating appetite, energy harvest,
74 gene expression, gut motility, and the conversion and storage of dietary energy (Chao et al., 2025;
75 Krajmalnik-Brown et al., 2012). Specifically, in T2D shifts in gut microbiome community and
76 functional composition have been compared to normal healthy subjects, which are in part alleviated by

77 treatment with the antidiabetic drug metformin (Forslund et al., 2015). Moreover, fecal microbiome
78 transplantation studies have demonstrated amelioration of clinical indicators of T2D (Chen et al., 2023;
79 Wu et al., 2023). Long-term diet is a key factor shaping gut microbiome composition (Gilbert et al.,
80 2018), and diet shaped by the gut microbiome can modify the expression and operation of intestinal
81 enteroendocrine cells (EECs) responsible for sensing and responding to nutrients (e.g., shifting from
82 serotonin to peptide producing EECs, or microbiome-dependent silencing of EECs; Aliluev et al.,
83 2021; Ye et al., 2019). These are multidirectional interactions, as loss of EECs and their secreted
84 hormones also changes microbiome structures (Blot et al., 2023).

85 Intriguingly, numerous assessments attribute a major share of T2D risk to environmental
86 exposures that extend well beyond lifestyle and dietary behaviors to include the built, natural, social,
87 and physicochemical environments (Beulens et al., 2022). Among these, strong population-level
88 evidence links T2D to a shift in living environments from rural to rapidly growing, densely populated
89 urban areas (Attard et al., 2012; Cheema et al., 2014; Gassasse et al., 2017; Ruiz-Alejos et al., 2018).
90 Importantly, microbiomes represent a key potential linkage mechanism between environmental
91 exposures consistent with T2D risk and metabolic processes occurring in the human gut. Gut
92 microbiomes are predominantly shaped by environmental factors, including diet and ambient
93 exposures, not host genetics (Rothschild et al., 2018). In particular, soils represent the globally
94 dominant source of environmental microbial exposures, providing home to 59% to >99.9% of species
95 on Earth (Anthony et al., 2023; Blakemore, 2025). The gut is dominated by spore-forming bacteria,
96 together with facultative anaerobes, capable of surviving and dispersing through aerobic environments
97 before re-establishing in suitable oxygen-deprived habitats (e.g., lower intestine, wet organic-
98 containing soil habitats) (Browne et al., 2016; Wyres & Holt, 2018). Butyrate-producing bacteria,
99 which ferment organic matter to produce the short-chain fatty acid butyrate and are considered key
100 members of gut microbiota, are found widely in many outdoor ecosystems and host organisms (Brame

101 et al., 2024). Exposure to naturally biodiverse soil microbiomes shapes and supplements gut
102 microbiome composition and functional capacity, particularly from a young age (Liddicoat et al., 2020;
103 Liu et al., 2021; Roslund et al., 2021; Wopereis et al., 2014; Zhou et al., 2024). Gut microbiota
104 variation in wild primates has been explained by environmental heterogeneity, especially soil
105 differences (Grieneisen et al., 2019). Consequently, soil microbes may be introduced to the gut
106 environment through direct soil contact, foodborne and airborne (e.g., dust) microbiomes.

107 Moreover, soil microbiome composition and functional capacity may shift in generalizable
108 ways with ecosystem quality (i.e., spanning anthropogenically disturbed/degraded to intact natural
109 condition), with potential to impact human health (Delgado-Baquerizo et al., 2021; Liddicoat et al.,
110 2024; Liddicoat et al., 2022). As urbanization accelerates—with around 70% of the global population
111 projected to live in cities by 2050 (UNDESA, 2019)—the degradation and homogenization of soil
112 microbiomes in urban and peri-urban environments may pose growing risks to human health (Sun et
113 al., 2023). Soil microbiomes are important for incorporating diverse nutrients into plant and animal-
114 derived foodstuffs including via microbial nutrient gathering (Cao et al., 2024), direct incorporation of
115 metabolites and components of microbial bodies (Adamczyk, 2021), and influencing gut microbiota of
116 ruminants (Qin et al., 2022). Therefore, soil ecosystem degradation may also impact on human gut
117 microbiomes and metabolism through dietary pathways. Exploring overlaps in the functional capacities
118 of (1) degraded versus naturally biodiverse soil microbiomes (linked to aboveground degraded versus
119 intact ecosystem condition) and (2) T2D versus normal healthy gut microbiomes, may reveal
120 previously unappreciated microbiome linkages between environments and T2D.

121 Despite their importance, the measurement and definition of 'healthy microbiomes' in humans
122 and soils remain challenging (Fierer et al., 2021; Van Hul et al., 2024). Approaches that examine the
123 range of functions performed by microbiomes are expected to offer improved understanding compared
124 to measures based on compositional diversity (Van Hul et al., 2024). Community-scale microbial

125 metabolism, where bioenergetic machinery and resources (including metabolites and electron transfer)
126 are shared in the extracellular space (Daisley et al., 2021), adds complexity and means that multiple
127 taxa may often combine to perform functional roles of interest. The need for improved insight into the
128 contributions of microbiomes in health and disease has motivated efforts to analyze soil and gut
129 microbiomes through the lens of their *compound processing potential* (CPP) (Liddicoat et al., 2024;
130 Figure S1). A compound-oriented approach can provide more direct functional information about
131 microbiomes because of known biochemical and health associations with chemical compounds (e.g.,
132 microbial feedstocks, substrates and metabolites, such as carbohydrates, lipids, proteins, amino-sugars,
133 vitamins), which are not described for most microbial taxa. New insights into differential compound
134 processing by microbiomes may also point to mechanistic hypotheses and/or possible therapeutic
135 targets. Microbial metagenomes (the entire collective DNA of a sample) are used to examine functions.
136 However, existing approaches require specialized knowledge of complex biochemical pathways and do
137 not provide resolution at the fundamental level of the chemical compounds involved (i.e., individual
138 compounds may often participate in multiple pathways). Existing metabolite prediction frameworks
139 (e.g., Garza et al., 2018; Mallick et al., 2019; Neveu et al., 2023) rely variously on metabolome training
140 datasets, microorganism-specific genome-scale metabolic models, taxonomic abundance estimates, and
141 assumed environmental conditions, to model short-term reaction rates. However, these approaches are
142 unsuited to highly biodiverse, poorly characterized and varying environments, such as soils (Liddicoat
143 et al., 2024). Thus, there is a need for new, compound-oriented methods to examine normal versus
144 aberrant functioning in soil and gut microbiomes.

145 Here we explore and compare model case study soil and gut metagenome datasets from
146 gradients of ecosystem quality and in subjects with T2D versus normal health by quantifying their
147 genomic potential to metabolize an array of chemical compounds. Specifically, the aims were to
148 improve functional and metabolic insights to the role of microbiomes in ecosystems and T2D, and their

149 potential overlaps, through identifying: (1) compounds with differential potential metabolism (i.e., with
150 trending CPP) based on analysis of soil metagenomes across an ecosystem quality gradient (i.e.,
151 degraded to intact natural condition); (2) compounds with differential potential metabolism (based on
152 CPP values) from gut metagenomes in untreated T2D versus normal health; and (3) overlapping
153 compounds and biochemical/functional pathways between (1) and (2) that may reveal new insights to
154 potential environmental microbiome contributions to T2D.
155

156 ## 2. Materials and Methods

157 ### 2.1. Case study data

158 We examined four model case study datasets (Table 1) comprising previously published (1) soil
159 metagenomes from a restoration gradient case study (that compares degraded post-mining, reforested,
160 and unmined reference sites; Sun & Badgley, 2019) and disturbed versus natural soils (as described in
161 Liddicoat et al., 2024) from the Australian Microbiome Initiative (AMI; Bissett et al., 2016); and (2)
162 gut metagenomes from subjects with untreated T2D and normal healthy controls (Forslund et al.,
163 2015). We determined CPP trends with restoration (described below), however, our subsequent focus
164 was to characterize potential metabolism outcomes associated with degraded ecosystems corresponding
165 to the more disturbed soil microbiomes in the younger revegetation age samples of the restoration
166 gradient. We used two T2D datasets comprising separate case study cohorts. The first T2D dataset
167 from Sweden (Karlsson et al., 2013) was analyzed to identify differences in CPP or potential
168 metabolism in T2D, and then assess overlaps with trending CPP found in more degraded (towards
169 younger) samples from the restoration gradient case study. A second T2D dataset from China (Qin et
170 al., 2012) was then integrated to confirm and consolidate trends in compound-level potential
171 metabolism that emerged consistently across the restoration gradient and both T2D case studies. Lastly,
172 corroboration of overlapping CPP trends was sought in the disturbed versus natural AMI soils dataset.

173

174 **Table 1.** Description of case study metagenome datasets.

| **Case study** | **Main comparison groups (and sample/subject numbers)** | **Metagenome functional profile characteristics (sample mean ± s.d.)** | **Source data reference; sequencing platform** | **Study nation of origin** |
|---|---|---|---|---|
| **Soil** | | | | |
| Post-mining forest ecosystem restoration ('restoration gradient') | Soil samples spanned revegetation ages of 6, 12, 22, 31 years, and unmined (UM) samples (treated as ordinal variables). Comprising five age-based groups, each with three replicates. Total *n* = 15 | Functions (SUPER-FOCUS): *n* = 30,125 (20,328 ± 767). Functional % all sum to 100%. Compounds (CPP profiling): *n* = 8,370 (7,777 ± 92). CPP (%): (67 ± 0.4 %) | MG-RAST project mgp16379 (Sun & Badgley, 2019); Illumina HiSeq | United States |
| Australian Microbiome Initiative (AMI) (Validation dataset) | Disturbed soils (*n* = 29) from agricultural land uses (cereals, cotton, horticulture, irrigated pasture) or natural soils (*n* = 55) from conservation and national park land uses. Total *n* =84 | Functions (SUPER-FOCUS): *n* = 37,335 (22,959 ± 1172). Functional % all sum to 100%. Compounds (CPP profiling): *n* = 8,515 (7,994 ± 127). CPP (%): (65.7 ± 1.2 %) | AMI Data Portal [Data accessed Sep 2022] (Bissett et al., 2016)[a]; Illumina HiSeq | Australia |
| **Human gut** | | | | |
| Type 2 diabetes (T2D) vs. Normal | T2D with no metformin treatment (T2D met-, *n* = 33), vs. normal healthy (*n* = 43). Subjects were females only, aged 69-72 years. Total *n* = 76 | Functions (SUPER-FOCUS): *n* = 17,912 (7833 ± 1699). Functional % all sum to 100%. Compounds (CPP profiling): *n* = 7,024 (5,287 ± 585). CPP (%): (70.5 ± 2.9 %) | SRA accession PRJEB1786 (Forslund et al., 2015; Karlsson et al., 2013)[b]; Illumina HiSeq | Sweden |
| T2D vs. Normal | T2D met- (n = 30; females *n* = 17, aged 51-71 years; males | Functions (SUPER-FOCUS): *n* = 19,352 (8650 ± 4214). | SRA accession PRJNA422434 | China |

| | | | | |
|---|---|---|---|---|
| (Validation dataset) | *n* = 13, aged 51-75 years). Normal (*n* = 52; females *n* = 24, aged 51-67 years; males *n* = 28, aged 52-74 years). Total *n* = 82 | Functional % all sum to 100%. Compounds (CPP profiling): *n* = 7,213 (5,310 ± 1670). CPP (%): (65.7 ± 4.1 %) | (Forslund et al., 2015; Qin et al., 2012) [b]; Illumina Genome Analyzer II | |

Note: [a]AMI Data portal URL: https://data.bioplatforms.com/organization/australian-microbiome; AMI soils were surface depth (0–10 cm), from the temperate climate zone, with 7.5–45 % clay content (i.e., avoiding very sandy and very clayey soils). [b]Forslund et al. (2015) applied metformin treatment data in a reanalysis of metagenome data from Swedish (Karlsson et al., 2013) and Chinese (Qin et al., 2012) cohorts. We analyzed a subset of each cohort. From the Swedish cohort, we excluded subjects who were treated with metformin or diagnosed with impaired glucose tolerance (prediabetes). From the Chinese cohort, used here for validation purposes, we only included subjects >50 years old to compare with the mature age (69-72 years) Swedish subjects.

### 2.2. Metagenome functional profiling via compound processing potential (CPP)

The CPP analysis approach uses a novel bioinformatics workflow that redistributes values of functional potential relative abundances (%), from the scale of functions (i.e., biochemical pathways that often comprise multiple linked chemical reactions), down to the level of the individual chemical compounds. These compounds are conceptually participating in the array of intersecting chemical reactions represented in the collective functional capacity of a metagenome. These methods do not measure actual chemical compounds. Rather, they quantify the genetic capacity of a microbial community to metabolize (or process) compounds, as written in the metagenome. The distinction is not made between compounds being consumed (reactants) or produced (products) as functional pathways may often involve strings of chemical reactions (metabolic processes), involving one or many organisms, where a compound can be the output of one reaction and an input to another.

Measures of CPP were derived following the steps outlined in Figure 1. Firstly, following Liddicoat et al. (2024), using high performance computing resources (Flinders_University, 2021; Pawsey_Supercomputing_Research_Centre, 2023) raw metagenomic sequences were accessed, trimmed for quality control, and human case study sequences that mapped (using Bowtie2; Langmead & Salzberg, 2012) to the human genome (GRCh38.p14 / hg38) were excluded (low levels were observed; Supplementary Material, Supporting Information). Then good quality read 1 sequences were classified to SEED subsystem (Overbeek et al., 2013) functional annotations and relative abundances using SUPER-FOCUS software (Silva et al., 2015). Every SUPER-FOCUS function (output row) was translated to one or more corresponding chemical reaction(s) using a purpose-built R-script algorithm based on ModelSEED database lookup tables (from https://github.com/ModelSEED/ModelSEEDDatabase; accessed 10 Aug 2022). Linking of functions (and sub-functions where present) to chemical reactions was based on either: full matching of functional hierarchies (using subsystem-class, -subclass, -name and -role); detection of Enzyme Commission (EC) number; or matching of SUPER-FOCUS function name within ModelSEED lookup tables for reactions (reaction name or alias), subsystems (role), or reaction-pathways (external reaction name). Reactions were then linked to corresponding compounds using ModelSEED database tables (‘reactions.tsv’, ‘compounds.tsv’). Advancing from the previous method used in Liddicoat et al. (2024), we derived CPP measures at the resolution of individual compounds (all software parameters and supporting R code for CPP calculations are available at: https://github.com/liddic/cpp3d_t2d):

a. Function level relative abundances from SUPER-FOCUS (which sum to 100% in each sample) were allocated to compounds by dividing values equally across all sub-functions and reactions, and dividing again across all corresponding compounds, with weightings to account for stoichiometric coefficients for each compound within each reaction, with equal consideration to reactant and product compounds.

b. Relative abundances (%) for respective unique compounds (which may arise across multiple reactions and functions/pathways) were then collated and summed to provide a summary CPP relative abundance (%) for each compound. (Here, conversion to CPP data format successfully recovered typically 65 to 70% of the initial 100% function potential per sample; Table 1).

c. To assist mapping visualization of CPP patterns for carbon-containing compounds, elemental ratios of O:C, H:C, and N:C were calculated. Such chemical mapping frameworks have been used previously to display and interpret groupings of energetically and chemically similar compounds (Liddicoat et al., 2024; Rivas-Ubach et al., 2018).

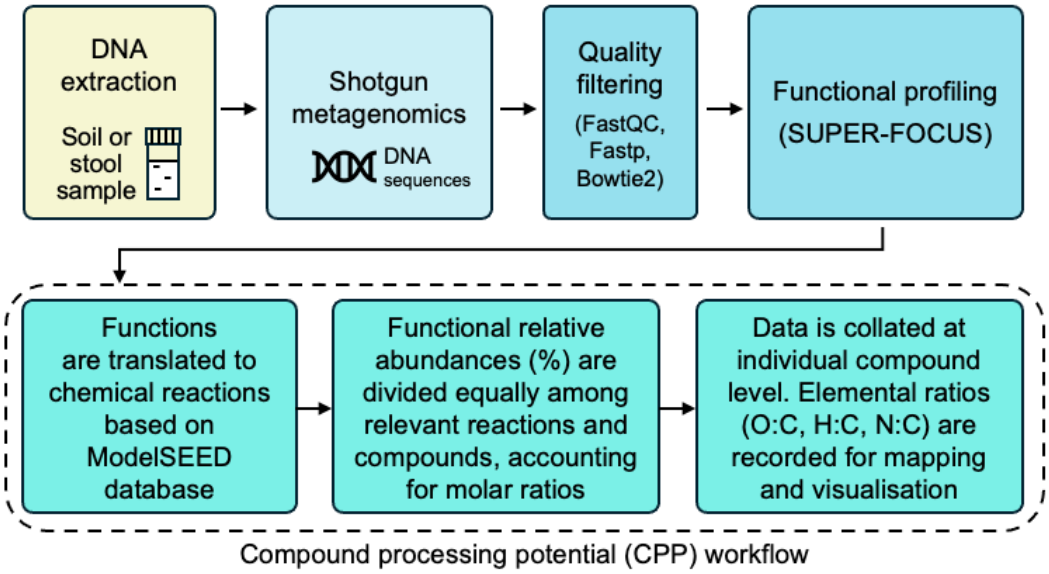



**Figure 1.** Overview of the CPP analysis workflow used to translate microbial metagenome functional potential relative abundances to quantify potential metabolism at the level of individual compounds.

### 2.3. Statistical Analysis

We used a staged approach to explore overlapping potential metabolism (i.e., CPP) trends in the soil and T2D gut samples, first examining the restoration gradient and lastly the AMI soils to corroborate findings (further detail is in Supplementary Material and at https://github.com/liddic/cpp3d_t2d):

236 a. ***Thirty selected compounds*** were chosen due to their relevance to soil and/or gut health (e.g.,
237 Blanco-Pérez et al., 2021; Daisley et al., 2021; Krajmalnik-Brown et al., 2012; Six et al., 2006;
238 compounds and reference information are listed in Table S1). For this list of compounds, CPP
239 trends were evaluated using Kendall's tau rank correlation tests based on ordinal revegetation
240 age classes. We interpreted CPP trends with ecosystem degradation as the inverse of calculated
241 trends with increasing revegetation age (towards intact natural condition). This assumption that
242 degradation may be modelled as the opposite of restoration is based on previous work showing
243 highly disturbed post-mining soil microbiomes progressively shift in their structure with
244 ecosystem restoration (Liddicoat et al., 2022). CPP trends (i.e., differences) with T2D versus
245 normal health were evaluated using non-parametric Wilcoxon rank-sum (Mann-Whitney) tests,
246 due to often differing variance of groups (T2D vs. normal) and to avoid normality assumptions.
247 All data were plotted to assist interpretation.
248 b. ***Compound-wide association tests*** were also performed to exhaustively scan all compounds
249 represented in the respective CPP datasets, using the same trend evaluation methods as above.
250 In soils, compounds that did not have relative abundance data for at least a quarter of the total
251 sample number were excluded due to low data. In restoration gradient soils, strong trends were
252 discovered, and only significant results based on adjusted *P*-values were considered further (i.e.,
253 after applying Benjamini and Hochberg false discovery rate correction). In the separate
254 compound-wide analysis of T2D gut samples, compounds were only included if at least one or
255 other of the T2D or normal groups contained at least 50% non-zero CPP values. Testing was
256 based on $\log_{10}$-transformed CPP % values, after zero replacement with small positive values
257 equal to half the minimum non-zero CPP % of all samples. One-sided (directional) difference
258 tests were based on whether the median value in T2D was greater or less than the median value
259 for normal healthy subjects. The T2D (Swedish cohort, SWE) Wilcoxon rank sum test results

260 yielded many *P*-values < 0.05. However, none were low enough to withstand Benjamini and
261 Hochberg *P*-adjustment for multiple testing, therefore, *P*-adjustment was not performed in the
262 compound-wide T2D gut data to retain the most interesting (potentially significant) results for
263 further exploration.

264 c. ***Overlap analysis.*** Compounds with CPP % trends in both the restoration gradient and T2D
265 (SWE) case studies were identified, based on matching ModelSEED compound identifiers. Our
266 focus was to highlight compounds that displayed consistent increases or decreases in CPP %
267 with degraded/disturbed ecosystems (i.e., towards younger revegetation age) and in T2D.

268 d. ***Visualising compound-wide association test results.*** Next, compounds with CPP % trends in
269 the restoration gradient and T2D (SWE) gut microbiomes, and overlaps between the two, were
270 mapped using their chemical formula elemental ratios of O:C, H:C, and N:C. From this
271 mapping space, prominent localized clusters of consistently trending compounds (i.e., with
272 ecosystem degradation and T2D) were identified and further analyzed to determine trends in
273 their collective (summed) CPP % and to interpret their possible metabolic importance.

274 e. ***Trend confirmation and consolidation.*** The above steps were repeated with the second T2D
275 (Chinese cohort, CHN) dataset, which ultimately refined the list of individual compounds with
276 parallel trends in CPP % across all datasets. Trend and contextual information for these
277 compounds was reported and visualised to represent the significance ($-\log_{10}$(*P*-value); as used in
278 genome-wide association study Manhattan plots) of their association with disturbed/degraded
279 ecosystems and T2D, and the number of functional themes (level 3 subsystems) they are linked
280 to. Here, data for T2D were visualised using mean results from the Swedish and Chinese cohort
281 datasets. To corroborate parallel CPP trends from the restoration gradient and T2D datasets,
282 CPP trends for identified compounds were then assessed in disturbed versus natural AMI soils.

f. ***Beta diversity ordinations.*** Supplementary analyses using the restoration gradient and Swedish cohort dataset were performed to examine community-scale compositional patterns and differences between sample groups and analysis approaches, comparing conventional functional potential (%) data (termed 'functions') versus CPP (%) data. This analysis used principal coordinate analysis (PCoA) ordinations based on Bray-Curtis distances, and testing for differences between groups via permutational analysis of variance (PERMANOVA) and beta dispersion testing for homogeneity of groupings using the vegan R package (Oksanen et al., 2020). These data are presented in the Supplementary Material, Supporting Information and Figure S2.

# 3. Results

## 3.1. Potential metabolism trends with ecosystem degradation

Analysis of CPP trends for 30 selected compounds (Table S1; Figures S3-S32) found increased potential metabolism with ecosystem degradation for (very strong evidence, $P < 0.001$) fructose (Kendall's tau ~restoration, $\tau = -0.761$), sucrose ($\tau = -0.782$), arabinose ($\tau = -0.844$), glucose ($\tau = -0.802$), xylose ($\tau = -0.761$) and chitin ($\tau = -0.782$); also (strong evidence, $P < 0.01$) galactose ($\tau = -0.617$), mannose ($\tau = -0.597$) and hydrogen sulfide ($\tau = -0.617$); and (moderate evidence, $P \leq 0.05$) glucosamine ($\tau = -0.514$) and arabinoxylan (hemicellulose) ($\tau = -0.453$). Conversely, we found decreased potential metabolism with ecosystem degradation for ($P < 0.001$) acetate ($\tau = 0.72$), carbon dioxide ($\tau = 0.844$) and lignin ($\tau = 0.802$); also ($P < 0.01$) glycogen ($\tau = 0.597$), butyrate ($\tau = 0.658$), trimethylamine N-oxide ($\tau = 0.576$) and trimethylamine ($\tau = 0.555$); and ($P \leq 0.05$) propionate ($\tau = 0.432$) and methane ($\tau = 0.473$).

Compound-wide ordinal correlation trend analyses identified 2,122 compounds with significant trends ($n$ = 757 increasing, $n$ = 1,365 decreasing with revegetation age; compound and trend data are in Supplementary Material files). Carbon-containing compounds were mapped using chemical formula ratios O:C, H:C and N:C (Figure 2; $n$ = 720 increasing, $n$ = 1,255 decreasing with revegetation age) to visualize clusters of compounds of similar chemical and energetic composition that trend together. We interpreted trends with ecosystem degradation as the opposite of the visualized trends with revegetation age (restoration). An example plot visualizing all carbon-containing compounds within a single sample is provided in Figure S33.

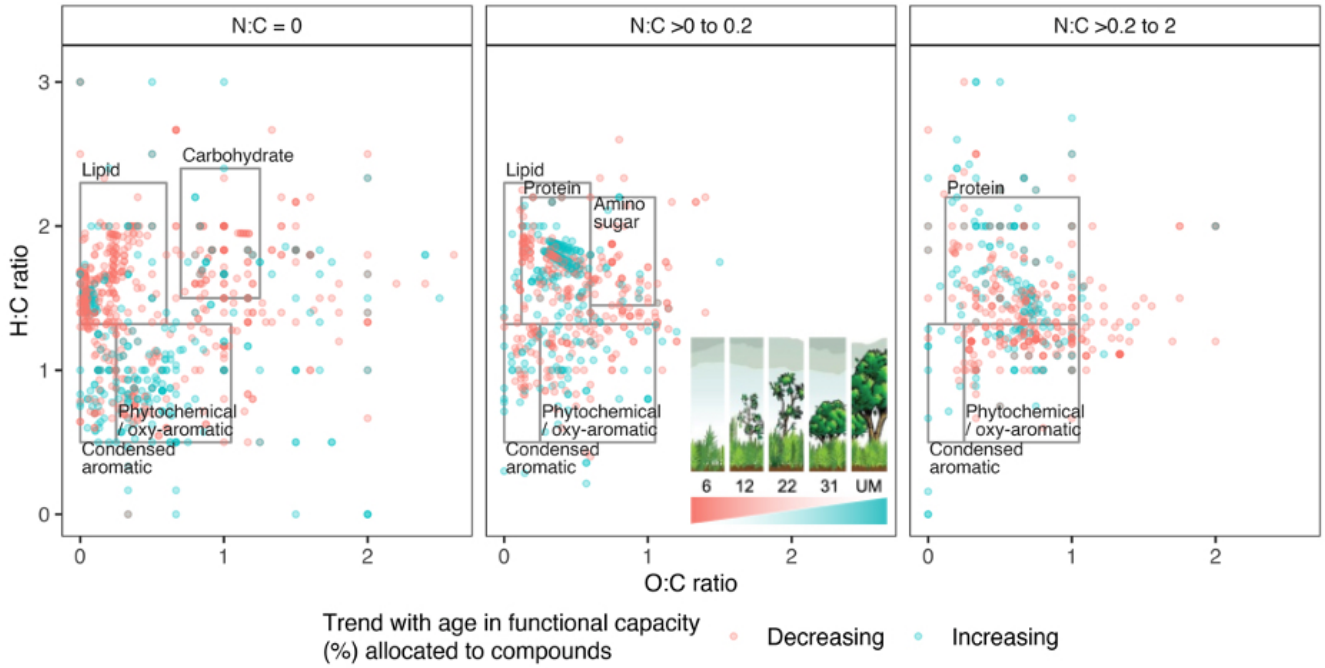



**Figure 2.** Compound-wide trend analysis results derived from soil metagenomes (described in Table 1), showing carbon-containing compounds with increasing (aqua, $n$ = 720) or decreasing (red, $n$ = 1,255) compound processing potential (CPP, i.e., functional capacity % or potential metabolism at individual compound scale) with revegetation age. Each point represents a compound, mapped via its chemical formula O:C, H:C and N:C elemental ratios. Only compounds that have trending potential

320 metabolism (i.e., shifting CPP % values) from degraded to mature/natural ecosystem quality are
321 displayed. Clusters of points indicate energetically and chemically similar compounds. The O:C by
322 H:C panels are zoomed in from their full extent to improve visualization of more data-rich regions. N:C
323 ratios are displayed as three depth-slices to represent the third dimension. Rectangular zones denoting
324 compound classes are provided as a visual guide only (adapted from Rivas-Ubach et al., 2018; Wu et
325 al., 2018).
326

327 **3.2. Potential metabolism trends in T2D**

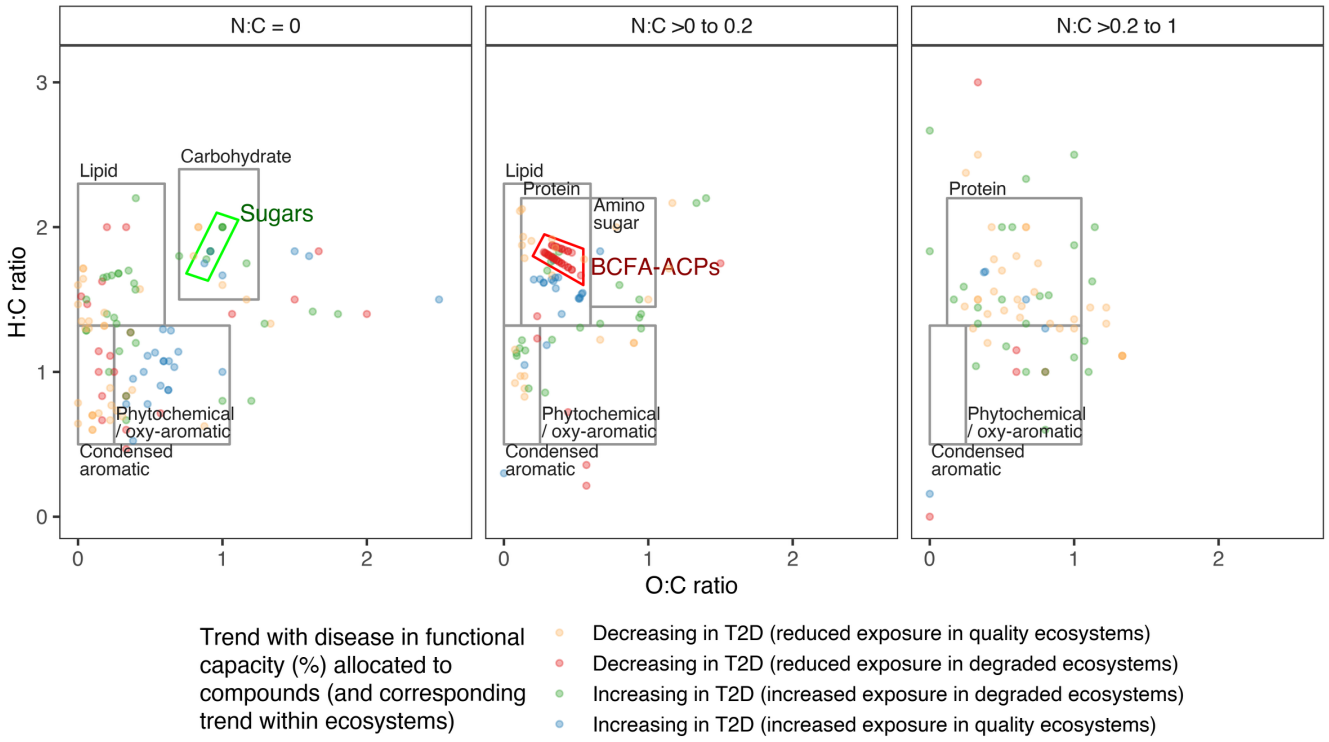
328 In the 30 selected compounds, we found consistent trends in the Swedish (SWE) and Chinese (CHN)
329 cohort datasets (Table S1; Figures S3-S32, S34) for increased potential metabolism in T2D for fructose
330 (SWE: $P \leq 0.05$, CHN: $P < 0.01$), arabinose (SWE: $P \leq 0.05$, CHN: $P < 0.001$), and amylopectin (SWE
331 and CHN: $P \leq 0.05$). We found decreased potential metabolism in T2D for lignin (SWE and CHN: $P \leq$
332 $0.05$).

333 Numerous trends were only found in one T2D cohort or the other. Increased potential
334 metabolism was found in T2D in the Swedish cohort for sucrose and starch ($P \leq 0.05$), and in the
335 Chinese cohort for mannose ($P < 0.001$); glucose, galactose and xylose (all $P < 0.01$). Decreased
336 potential metabolism was found in T2D in the Chinese cohort for acetate, propionate, butyrate, carbon
337 dioxide, hydrogen sulfide, indole (all $P < 0.001$); Menaquinone (Vitamin K2), glucosamine ($P < 0.01$);
338 methane, p-cresol and serotonin (all $P \leq 0.05$). We found conflicting potential metabolism trends for
339 ammonia across the cohorts, which increased in Swedish ($P < 0.01$) but decreased in Chinese ($P <$
340 $0.001$).

341 The compound-wide Wilcoxon rank sum trend analyses in T2D Swedish and Chinese cohorts
342 identified similar numbers of compounds with increased potential metabolism, but substantially higher
343 numbers of compounds with decreased potential metabolism in the Chinese cohort (visualized in

344 Figures S35 and S36; compounds and trend data for both T2D datasets are available in Supplementary
345 Material). For consistency, across both compound-wide T2D association analyses, and to retain the
346 most interesting T2D (SWE) cohort data, these data were not subject to *P*-adjustment for multiple
347 testing. In the T2D (SWE) data, a total of 1269 compounds with trending potential metabolism (n =
348 911 decreased, *n* = 358 increased) were found. From these, a total of 1181 carbon-containing
349 compounds (*n* = 860 decreased, *n* = 321 increased) were mapped using chemical formula ratios O:C,
350 H:C and N:C (Figure S35). For the T2D (CHN) data a total of 4292 compounds (*n* = 3984 decreased, *n*
351 = 308 increased) were identified, corresponding to 3964 carbon-containing compounds (*n* = 3695
352 decreased, *n* = 269 increased) mapped using chemical formula ratios O:C, H:C and N:C (Figure S36).
353 From visual inspection (comparing Figures S35 and S36), the chemical ratio mapping displays some
354 zones of similarity (e.g., in the vicinity of carbohydrates) but also dramatic differences due to the
355 widespread number of compounds with reduced potential metabolism in the T2D (CHN) dataset.
356
357 **3.3. Consistent CPP trends with ecosystem degradation and T2D**
358 Within the list of 30 selected compounds (Table S1), we found limited cases of consistent potential
359 metabolism trends across all three datasets – i.e. with ecosystem degradation, T2D (SWE), and T2D
360 (CHN). Specifically, we found increased potential metabolism in fructose and arabinose and decreased
361 potential metabolism in lignin was consistent across all datasets (Table S1).
362 Using the compound-wide trend results from T2D (SWE) and restoration gradient datasets, we
363 performed an overlap analysis, based on matching ModelSEED compound identifiers, to highlight
364 compounds with potential metabolism trends across both case study scenarios (compound and trend
365 data are in the Supplementary Material). From a total of 304 compounds that had CPP trends with T2D
366 (SWE) and across the restoration gradient, 286 carbon-containing compounds were visualized through
367 chemical ratio mapping (Figure 3). Decreased CPP (i.e., potential metabolism) with T2D (SWE) and

368 ecosystem degradation was observed in 70 compounds (66 contained carbon; red dots in Figure 3).
369 Increased CPP with T2D (SWE) and ecosystem degradation was observed in 89 compounds (81 with
370 carbon; green dots in Figure 3). Matching trends with ecosystem restoration were also found.
371 Decreased CPP with T2D (SWE) and ecosystem restoration was observed in 95 compounds (90
372 contained carbon; orange dots in Figure 3). Increased CPP with T2D (SWE) and ecosystem restoration
373 was observed in 50 compounds (49 with carbon; blue dots in Figure 3).
374




375

376 **Figure 3.** Overlap analysis showing carbon-containing compounds with trending potential metabolism
377 in both compound-wide trend analyses of the T2D (Swedish cohort) and restoration gradient case study
378 datasets (i.e., this plot shows overlapping compounds from Figures 2 and S35). Clusters of each color
379 represent energetically and chemically similar compounds occurring in each of the four trend groups
380 listed (numbers of compounds are provided in the text). Further description of the plot format is

381 provided in Figure 2. Expanded views of the grouped 'sugars' and branched-chain fatty acid - acyl-
382 carrier proteins (BCFA-ACPs) are provided in Figures S37-S41.
383
384 With our focus on microbiome functional shifts with ecosystem degradation, and to further
385 explore compounds that also shared potential metabolism trends with T2D (SWE), we inspected the
386 mapping visualization in Figure 3 and observed two prominent clusters of compounds with matching
387 trends (also see Table S2-S3, Figures S37 and S39). The first group outlined in green and labelled
388 'Sugars' in Figure 3 comprised six carbohydrate compounds (L-arabinose, melibiose, manninotriose,
389 D-lyxulose, sucrose, D-fructose; Table S2) that all display increasing CPP in both T2D (SWE) and
390 with ecosystem degradation. In the O:C, H:C, N:C mapping space, these sugars all occur on the line
391 H:C = 2 × O:C, with N:C = 0 (i.e., green points in Figure S37). Analysis of summed CPP values for
392 these sugars showed that they collectively exhibit a very strong increase with ecosystem degradation
393 (Kendall's tau ~restoration = -0.844, $P$ = <0.001) and a strong increase in T2D (SWE) (Wilcoxon rank
394 sum W = 985, $P$ = 0.002) (Figures 4 and S38).
395 The second group outlined in red in Figure 3 comprised 35 unique compounds involved in fatty
396 acid biosynthesis. This group was labelled BCFA-ACPs (branched-chain fatty acid – acyl-carrier
397 proteins) as it is dominated by this type of compound ($n$ = 30), together with $n$ = 5 straight-chain fatty
398 acid-ACPs (Table S3). All compounds in the BCFA-ACPs group have reduced CPP in T2D (SWE) and
399 reduced CPP in degraded ecosystems, and they each occur on two straight lines in the O:C, H:C, N:C
400 mapping space (line 1: H:C = -0.625 × O:C + 2, and line 2: H:C = -0.375 × O:C + 2, where N:C is in
401 the range >0 to 0.2; Figure S39). Analysis of summed CPP values for the BCFA-ACPs group showed
402 that they collectively exhibited a very strong decrease with ecosystem degradation (Kendall's tau
403 ~restoration = 0.741, $P$ = <0.001) and also moderate reduction in T2D (SWE) (Wilcoxon rank sum W
404 = 518, $P$ = 0.023) (Figures 4 and S40). When the summed CPP data for these groups were validated in

the T2D Chinese cohort data, they showed very strong agreement (sugars: Wilcoxon rank sum W = 1149, $P$ <0.001, BCFA-ACPs: W = 335, $P$ <0.001; Figure 4, detailed plots in Figure S41).

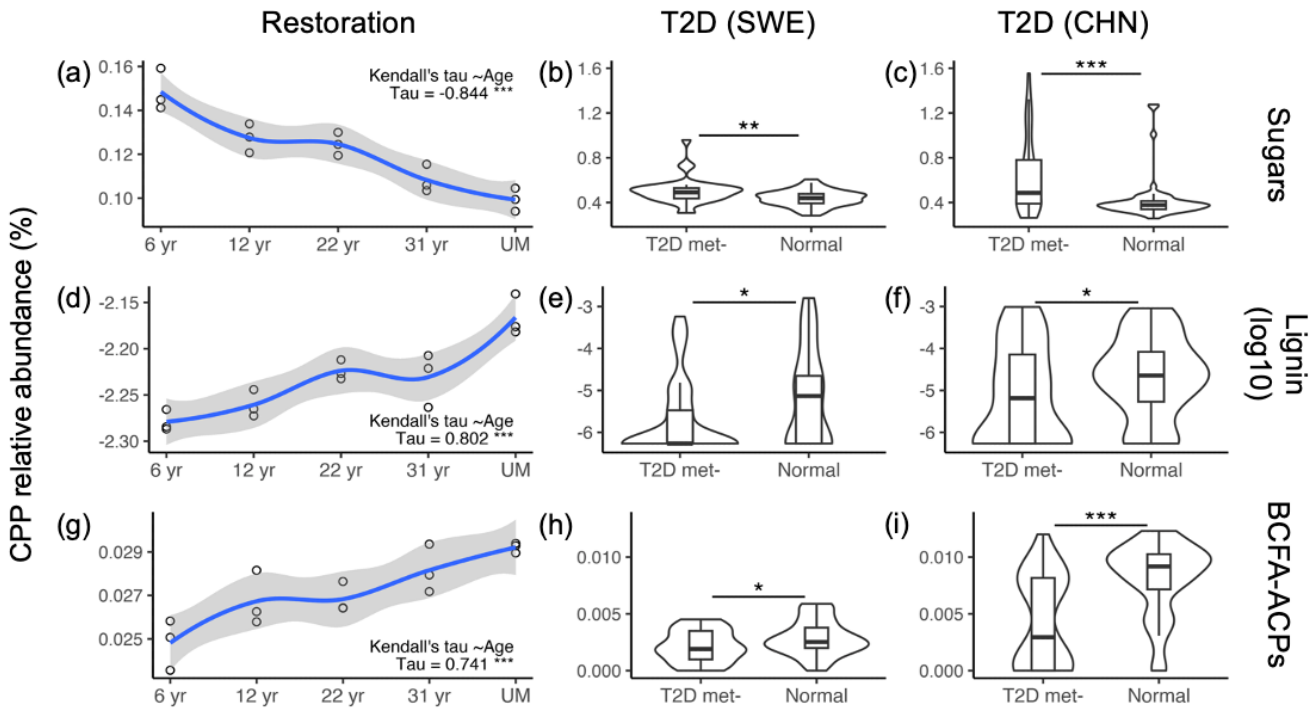



**Figure 4.** Potential metabolism of sugars (a-c), lignin (d-f), and branched-chain fatty acid – acyl carrier proteins (BCFA-ACPs, listed in Tables S3, S4) (g-i), within the restoration gradient (spanning restoration planting ages 6, 12, 22, 31 years and unmined, UM) (a, d, g), T2D (Swedish cohort, SWE; T2D met- = type 2 diabetes not treated with metformin, Normal = normal healthy subjects) (b, e, h), and T2D (Chinese cohort, CHN) (c, f, i). Sugars and BCFA-ACPs were groupings identified via matching trends in T2D (SWE) and with ecosystem degradation (Figure 3). Sugars were graphed as the sum of compound processing potential (CPP, %) for six compounds (L-arabinose, melibiose, D-fructose, sucrose, manninotriose and D-lyxulose), however subsequent testing showed only three of these sugars (L-arabinose, melibiose, D-fructose) displayed individual trends in T2D (Chinese cohort, CHN) (Tables S2, S4). Lignin CPP values were $\log_{10}$-transformed to improve display.

419 To confirm and consolidate individual compound trends exhaustively across all compound-wide
420 trend results, we further isolated only those ModelSEED compound identifiers with matching trends
421 across the restoration gradient and T2D case studies. This process identified 76 compounds with $n = 60$
422 decreased, $n = 16$ increased with ecosystem degradation, T2D (SWE) and T2D (CHN) (Figure 5; Table
423 S4; compound and trend data are further detailed in Supplementary Material). Summary trend and
424 contextual information are displayed in Figure 5, using mean results from T2D (SWE) and T2D (CHN)
425 cohort datasets. From this filtering process at the individual compound level, remarkably, the signal of
426 decreased potential metabolism for all 35 compounds in the BCFA-ACPs group previously identified
427 from overlapping restoration gradient and T2D (SWE) datasets also remained when intersected with
428 the T2D (CHN) dataset.

429 The BCFA-ACPs group comprised three categories of compounds: monomethyl unsaturated
430 medium to long BCFA-ACPs ($n = 18$), monomethyl saturated predominantly medium BCFA-ACPs ($n$
431 $= 12$), and unsaturated short, medium, and long straight-chain fatty acid-ACPs ($n = 5$) (Table S3).
432 BCFA lengths are based on the main carbon chain (short: < 6, medium: 6-12, long: 13-21 carbon
433 atoms; Mao et al., 2023). All 35 compounds, for all case studies, were associated with NADH and
434 NADPH enoyl-ACP reductases (EC 1.3.1.9 and EC 1.3.1.10 respectively), while several compounds
435 from the restoration case study were also associated with beta-ketoacyl-ACP synthase (EC 2.3.1.41) –
436 all of which are involved in type II fatty acid biosynthesis. One compound from the BCFA-ACPs group
437 (unsaturated medium-length straight-chain (2E)-Decenoyl-[acp]) was also associated across all case
438 studies with trans-2-cis-3-decenoyl-ACP isomerase (EC 5.3.3.14), which is involved in unsaturated
439 fatty acid biosynthesis.

440 As monomethyl BCFA-ACPs dominated this trend group, we were curious to understand
441 whether they might feature there exclusively, or if similar compounds were also present throughout the
442 data and not display trends. We found hundreds of other unique non-trending ACP-containing

443 compounds (*n* = 296 restoration, *n* = 289 T2D-SWE, *n* = 293 T2D-CHN), spanning a multitude of
444 enzymes or sub-functions (*n* = 223 restoration, *n* = 137 T2D-SWE, *n* = 146 T2D-CHN). Focusing only
445 on the methyl-containing variants, a shared inventory of non-trending ACP compounds was found
446 across all three datasets. Intriguingly, there were no other monomethyl unsaturated (-enoyl) BCFA in
447 the datasets beyond those that featured in our BCFA-ACPs trend group. The remaining shared non-
448 trending methyl-containing ACP compounds (*n* = 56) either contained carbonyl (-oxo-) groups (*n* =
449 18), or hydroxyl (-hydroxy-) groups (*n* = 22), or ester groups (*n* = 9), while the remaining (*n* = 7) were
450 saturated BCFA-ACPs (one short chain, and six long 13-16 chain length). These latter, non-trending
451 saturated BCFA-ACPs also differed from the saturated BCFA-ACPs in our trend group, which
452 contained mostly medium-length chains (Table S3).

453 Additionally, when results from the T2D (CHN) dataset were intersected, this refined the results
454 for the sugars that were previously highlighted from overlapping the restoration gradient and T2D
455 (SWE) datasets. When the restoration gradient, T2D (SWE), and T2D (CHN) datasets were intersected,
456 arabinose, fructose, and melibiose still remained as trending, together with 6-phosphosucrose, an
457 intermediate in sucrose biosynthesis.

458 Corroboration of the observed overlapping trends was found in the Australian soil metagenomes
459 dataset. In disturbed AMI soils, we observed increased potential metabolism of L-Arabinose, D-
460 Fructose, Melibiose and 6-Phosphosucrose, and decreased potential metabolism of lignin and all 35 of
461 the BCFA-ACPs (all $P = <0.001$; Figure S42). From the 76 compounds with matching potential
462 metabolism trends in degraded soils (restoration gradient), T2D (SWE) and T2D (CHN), a total of 65
463 compounds (85.5 %) displayed shared potential metabolism trends in the AMI disturbed soils (Table
464 S4).
465

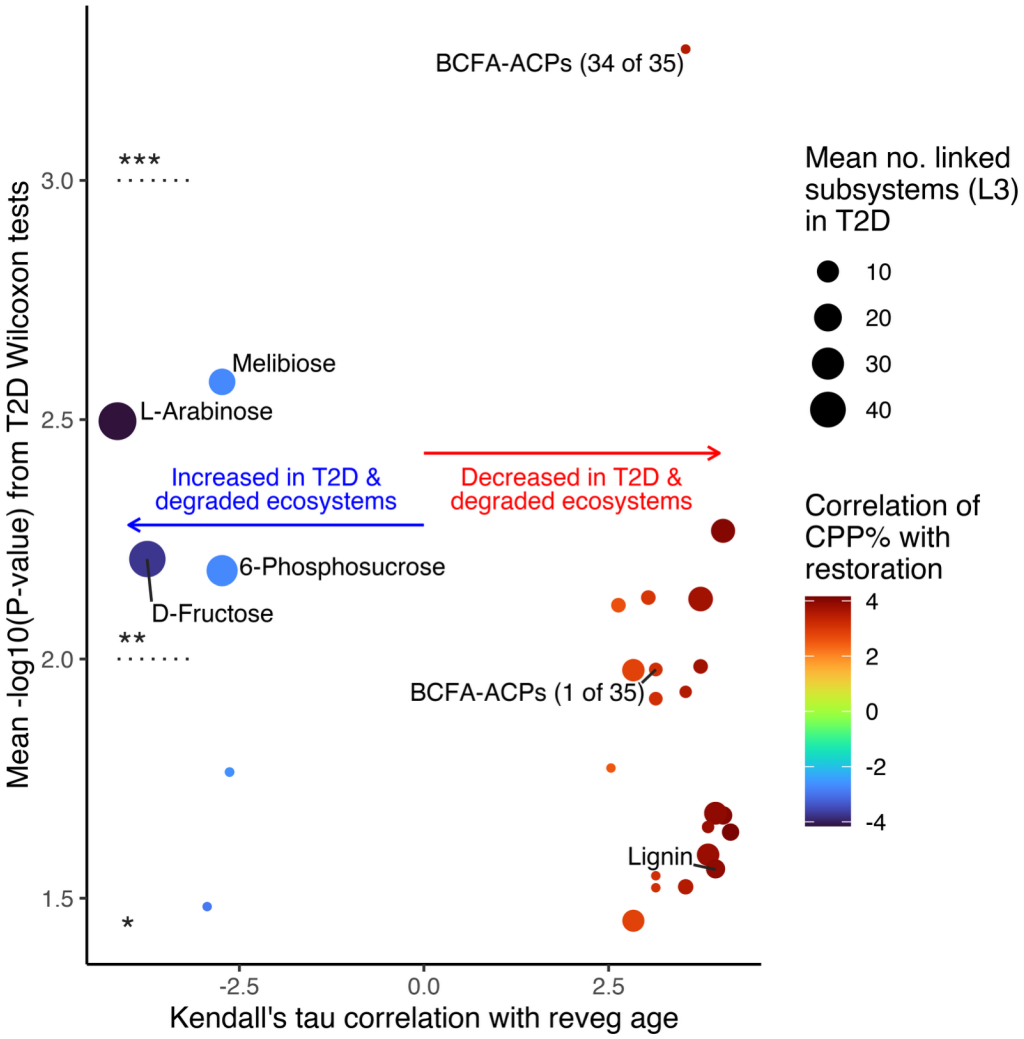



**Figure 5.** Relative importance of compounds with consistent trends across restoration gradient and T2D case study data, showing increased or decreased compound processing potential (CPP %, i.e., potential metabolism) with ecosystem degradation (soil) and in T2D (gut) microbiomes. Compounds including branched-chain fatty acid-acyl carrier proteins (BCFA-ACPs), sugars that feature widely across many functional themes and pathways, and lignin are highlighted in the plot. Trends with ecosystem degradation are interpreted as the inverse of restoration (based on Kendall's tau ordinal correlation with revegetation age classes). Measures of significance (y-axis) from the Wilcoxon difference testing of CPP in T2D (versus normal), and the number of level 3 (L3) subsystems

475 (functional themes) linked to each compound, are calculated as mean values from the Swedish and
476 Chinese cohorts. Further details for compounds (*n* = 76 total) are in Table S4.
477

478 ## 4. Discussion

479 We identify several meaningful overlapping trends in the functional capacity of soil and human gut
480 microbiomes, supporting our hypothesis that increased population exposure to degraded soil
481 ecosystems, and/or nutrient-depleted (e.g., low BCFA content) food derived from them, may contribute
482 to the development of type 2 diabetes. These linkages warrant further investigation due to the rising
483 prevalence and impact of T2D and the widespread and increasing processes of global change impacting
484 soil microbiomes, which provide a key reservoir and source for priming and resupplying the human gut
485 microbiome. A possible alternative or complementary explanation may be that similar ecological
486 stresses and dysfunction in soil and gut ecosystems may be driving convergence in microbial functional
487 traits. Using a novel method, we translated community-scale microbial metagenome functional
488 capacities to quantify potential metabolism for individual compounds. This compound-oriented
489 approach facilitates linkages to a wealth of published literature that links chemical compounds with
490 metabolism, health and wider biological processes in humans and the environment. Our mapping of
491 compounds into the bioenergetically informed O:C, H:C and N:C coordinate space helped to reveal
492 meaningful patterns of simultaneously trending compounds (e.g., sugars, BCFA-ACPs) with soil-
493 ecosystem quality and T2D versus normal health.
494

495 ### 4.1. Do branched-chain fatty acids connect healthy ecosystems and healthy people?

496 Our data suggest that microbiomes in degraded ecosystems and T2D have reduced capacity for
497 monomethyl BCFA (mmBCFA) biosynthesis. This may reflect a shift in the biochemical composition
498 of bacterial assemblages as BCFAs are key components that enhance bacterial membrane fluidity, and

499 BCFA synthesis capacity enables membrane fluidity-related adaptive responses for survival and
500 proliferation through environmental stresses (Gozdzik et al., 2023; Yoon et al., 2015). BCFA levels
501 vary among bacterial taxa, being commonly higher in Gram-positive bacteria, present in some Gram-
502 negative species, and especially high in *Bacillus* and *Bifidobacterium* (Yang et al., 2025; Yoon et al.,
503 2015). Within ecosystems, BCFAs are present in lipid/waxy plant compounds, animal (including
504 human) tissues and fluids, including ruminant products (milk, cheese, meat), fish, organs, blood serum,
505 skin, hair, adipose tissue, colostrum, breast milk, *vernix caseosa* of newborns, and at very high levels in
506 saprotrophic fungi such as *Conidiobolus* spp. found in soil and decaying organic matter (Gozdzik et al.,
507 2023; Maheshwari et al., 2020; Mao et al., 2023). Therefore, variation in BCFA synthesis capacity
508 could result from changes in microbial taxonomic composition as ecosystems degrade.

509 Monomethyl BCFAs are of interest because they possess multiple beneficial bioactivities for
510 human health and provide a direct link between bacterial and human physiology (Mao et al., 2023).
511 Beneficial bioactivities of mmBCFA include lipid-lowering, reducing the risk of metabolic disorders,
512 maintaining normal β cell (i.e., insulin-producing) function and insulin sensitivity, anti-inflammation,
513 cytotoxicity to cancer cells and regulation of development (Gozdzik et al., 2023; Mao et al., 2023;
514 Yang et al., 2025). Bacterially-derived mmBCFAs facilitate the direct biochemical crosstalk (i.e.,
515 uptake and cross-domain metabolism) between the gut microbiome and animal hosts (Brown et al.,
516 2023; Zhu et al., 2013; Zhu et al., 2021). In the nematode *Caenorhabditis elegans* and mammalian
517 tissue culture cells, mmBCFA have been shown to trigger the host mechanistic target of rapamycin
518 complex 1 (mTORC1) (Zhu et al., 2013; Zhu et al., 2021), a central regulator of cell growth and
519 metabolism (Takahara et al., 2020). The host mTORC1 responds to environmental signals (e.g., amino
520 acid availability, growth factors, energy levels, stress) to coordinate cellular status. However, mTORC1
521 dysregulation is closely linked to diabetes, cancer, and neurodegenerative disorders (Takahara et al.,
522 2020). Liu et al. (2017) reported high capacities for human fetal intestinal epithelial cells (enterocytes)

523 to incorporate long-chain BCFA into membrane phospholipids. Following uptake by human intestinal
524 epithelial cells, further potential interactions between BCFA and host endogenous BCFA synthesis and
525 metabolism may occur (Gozdzik et al., 2023; Mao et al., 2023), including post-translational
526 modifications to cell proteins, which alter intestinal cell function (Ezzine et al., 2022). While the
527 precise mechanisms are uncertain, it is plausible that mmBCFA in the gut microbiome might modulate
528 host cellular metabolism and physiological functioning via these interactions.

529 Microbial sources play a dominant role in the supply of health-associated mmBCFA (Mao et
530 al., 2023; Yang et al., 2025). Key inputs to BCFA synthesis include branched-chain amino acids (which
531 can only be made in bacteria, plants, and fungi, but not in animals; Neinast et al., 2019) or shorter
532 BCFA, which can be elongated. Microbes (e.g., rumen bacteria) also contribute to BCFA synthesis in
533 animals (Mao et al., 2023). Subsequently, both diet and gut microbiome synthesis provide major
534 sources of BCFA to humans (Mao et al., 2023; Yang et al., 2025). Within microbial environments (e.g.,
535 in soil or the gut), the interchange of BCFA between substrates and microbes can be expected because
536 fatty acids are a high-value energy source, and bacteria can assimilate environmental BCFA into their
537 bodies (Sen et al., 2016; Yao & Rock, 2015). In the gut, the constant release of a portion of mmBCFAs
538 from bacterial cell membranes occurs due to the rapid turnover of bacterial cells from competitive
539 interactions (Booth et al., 2023) and the secretion of fatty acids and their derivatives (Brown et al.,
540 2023). A portion of these gut bacteria may be supplied from external ecosystem exposures (Roslund et
541 al., 2021; Rothschild et al., 2018) and be further shaped by diet and other factors (e.g., high BCFA-
542 content Bifidobacterium spp. proliferate in infants receiving breast milk; Stewart et al., 2018).

543 As ecosystems degrade, there may be several reasons why BCFA-synthesizing organisms
544 decline. Ecosystem degradation is widely associated with reductions in soil organic matter (e.g., Chen
545 et al., 2018; Cunningham et al., 2015; IPBES, 2018; Kooch et al., 2022). Indeed, the more degraded
546 soils from the restoration gradient case study dataset had lower soil organic content than unmined sites

547 (Avera et al., 2015). Soil organic matter comprises a continuum of progressively decomposed organic
548 compounds originating from plant residues, root exudates, microbial synthesis, and other chemical
549 reactions, and commonly includes amino acids and fatty acids (Song et al., 2024). Amino acids can be
550 released from proteins in the soil through proteolysis at rates correlated with soil organic matter pools
551 and soil protein concentrations (Näsholm et al., 2009). This release increases with ecosystem
552 succession (Kielland et al., 2007). Natural decomposition and recycling networks of saprotrophic fungi
553 (which includes many high BCFA-content spp.) may also be degraded, likely reducing BCFA content
554 and turnover in soil microbiomes. Diverse plant litter contributes to increased soil carbon, higher
555 microbial abundance, diversity, decomposition and turnover, which strengthens over time (Zhang et al.,
556 2023). In contrast, urban areas exhibit dramatically reduced fungal abundance and diversity compared
557 to natural ecosystems (Abrego et al., 2020). Urbanization has been associated with the homogenization
558 of soil microbiomes (Delgado-Baquerizo et al., 2021), which in turn reduces the diversity of
559 assemblages that resupply the human gut microbiome. Taken together, these influences may reduce
560 access to and recycling of both branched-chain amino acids and fatty acids for soil microbiomes in
561 degraded ecosystems, thereby potentially reducing ambient human exposure to health-promoting
562 microbial BCFA synthesis capacity (Figure 6). Degraded agricultural soils might also produce crops
563 and ruminant products (e.g., meat, dairy) with reduced BCFA content, that impact gut microbiomes via
564 diet, however these ideas require further investigation.
565

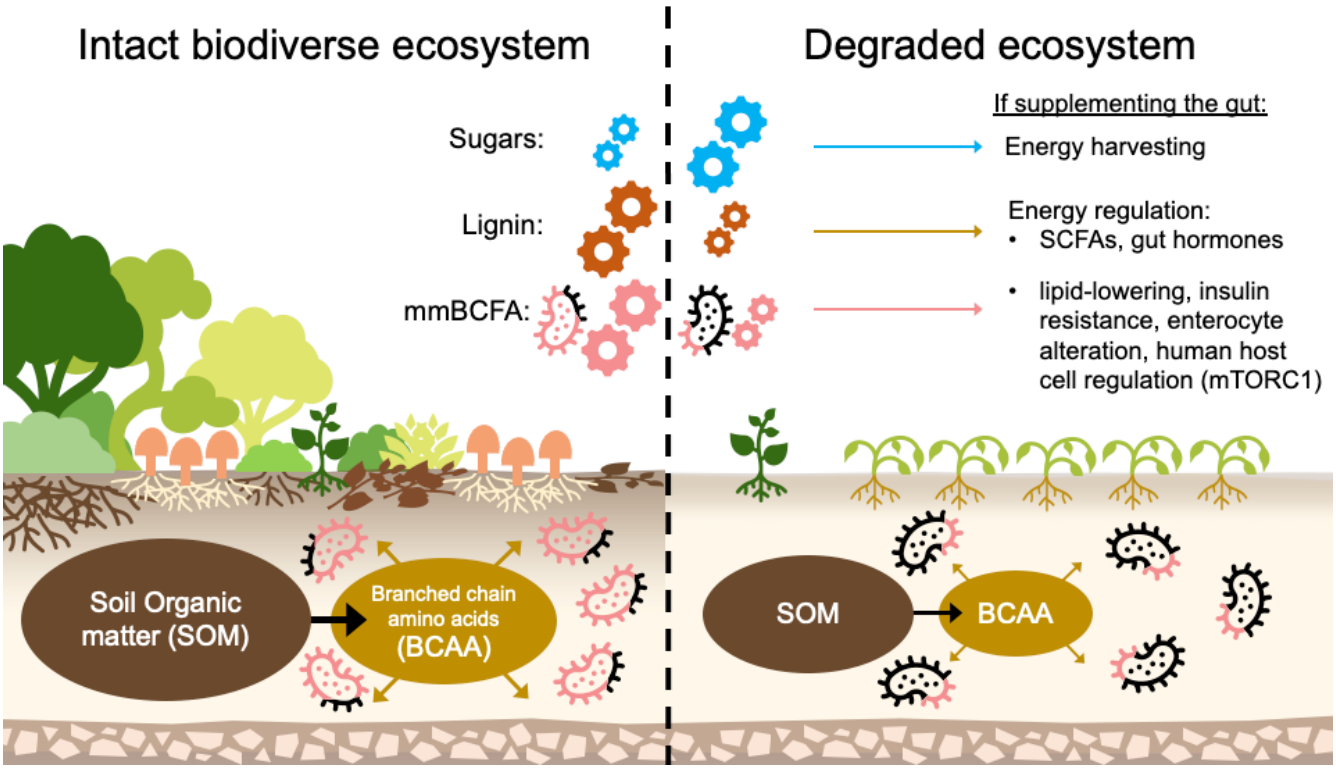



**Figure 6.** Hypothesis: Intact natural ecosystems typically have greater biodiversity, woody plant debris (including lignin), fungal recycling and soil organic matter (SOM) pools. SOM releases branched-chain amino acids (BCAA) which are used to make precious monomethyl branched-chain fatty acids (mmBCFA) used in bacterial cell membranes, stored by fungi, and needed to regulate animal cell metabolism via the mechanistic target of rapamycin complex 1 (mTORC1) among other pathways. With ecosystem degradation, soil organic matter decomposes into simple sugars. Degraded soil microbiomes have increased capacity to metabolise simple sugars, decreased capacity to metabolise lignin (potentially impacting production of short-chain fatty acids, SCFAs), and decreased mmBCFA synthesis capacity (potentially linked to mmBCFA content in microbial bodies and the wider ecosystem). If people are increasingly exposed to degraded soil microbiomes, and/or consume low mmBCFA-content food derived from these systems, it may contribute to metabolic shifts in the gut consistent with type 2 diabetes.

580 **4.2. Identified compounds linked to energy harvesting**

581 We found that potential metabolism of sugars (including arabinose, fructose, and melibiose) and the
582 intermediate compound 6-phosphosucrose each increased with ecosystem degradation and T2D.
583 Additionally, the potential metabolism of sucrose, glucose, galactose, xylose and mannose increased in
584 more degraded ecosystems. Sugars are the most abundant organic compounds in the biosphere and are
585 key carbon and energy sources for soil microbes (Gunina & Kuzyakov, 2015). Sugars in soil can either
586 be derived from primary sources (e.g., decomposition of plant litter, rhizodeposits, root exudates) or
587 secondary sources (e.g., microorganisms and their residues) (Gunina & Kuzyakov, 2015). Our
588 detection of increased potential metabolism of simple sugars (largely monosaccharides and
589 disaccharides) likely reflects enhanced microbial decomposition of soil organic matter within more
590 degraded, less biodiverse ecosystems (Chen et al., 2018). Previous work suggests that galactose,
591 glucose, mannose, arabinose and xylose are chief components of the estimated 5% sugar residues
592 contained within soil organic matter (Nagar, 1962). We might therefore conclude that increased
593 potential metabolism of sugars is indicative of microbiomes in degraded ecosystems that consume,
594 rather than build or maintain, soil organic matter.

595 In the gut microbiome, carbohydrate metabolism contributes up to 10% of the host's overall
596 energy extraction and is recognized as an important contributor to insulin resistance and the
597 development of obesity and prediabetes (Takeuchi et al., 2023). Takeuchi et al. (2023) found increased
598 gut microbiome metabolism of monosaccharides, including fructose, galactose, mannose and xylose,
599 significantly correlated with insulin resistance. Interestingly, in soils and humans, enhanced sugar
600 metabolism is also associated with more opportunistic bacteria (Le Bouguénec & Schouler, 2011).
601 Increased relative abundances of opportunists (including fast-growing, potential pathogens) have been
602 associated with disturbance in urban greenspace soils (Delgado-Baquerizo et al., 2021) and in human-
603 altered land uses (Liddicoat et al., 2019). If microbial assemblages with enhanced sugar metabolism are

604 transferred from degraded ecosystems to the human gut, they may exhibit opportunistic or pathogen-
605 like traits—potentially contributing to increased energy harvest and metabolic imbalances associated
606 with T2D.

607

608 **4.3. Identified compounds linked to energy management**

609 We found altered potential metabolism for compounds with plausible links to energy management
610 (e.g., energy storage via fatty acids, gut hormone secretion and appetite regulation) that decreased in
611 both degraded ecosystems and T2D. This finding supports the notion that exposures to degraded
612 ecosystem soils that are deficient in potential metabolism of compounds involved in energy
613 management may supplement and shape gut microbiomes that are also deficient in this way, thereby
614 contributing to further metabolic imbalances found in T2D. We discuss above how gut microbiome
615 mmBCFA content and biosynthesis capacity might modulate host cellular metabolism via central
616 mTORC1 regulation, alteration of enterocyte phospholipid composition and function, and/or supply of
617 BCFA for further host endogenous fatty acid synthesis and BCFA-mediated metabolism.

618 Dietary fiber, such as lignin, is known to decrease plasma ghrelin, known as the hunger
619 hormone, and increase other key gut regulatory hormones: cholecystokinin (CCK), glucagon-like-
620 peptide-1 (GLP-1), and peptide YY (PYY) (Bulsiewicz, 2023). Short-chain fatty acids (SCFAs) are
621 produced when certain members of the gut microbiome ferment non-digestible fibers, and these SCFAs
622 bind to receptors in the colon, leading to the production of a range of key hormones that regulate food
623 intake, body weight, and energy metabolism (Bulsiewicz, 2023; Chao et al., 2025). We included both
624 lignin and pectin to represent fiber, which were detected in the datasets and are among the recognized
625 key types of dietary fibers (Blanco-Pérez et al., 2021). Potential metabolism of lignin decreased with
626 ecosystem degradation and in T2D gut microbiomes. Similar trends appeared for sinapyl alcohol and
627 coumaryl alcohol (both precursors to lignin) in the compound-wide results spanning all datasets.

628 Potential metabolism for pectin also decreased in T2D (significantly in the Swedish cohort, and
629 marginally in the Chinese cohort), but not in degraded ecosystems. We also searched for
630 oligosaccharides (e.g., fructan, inulin, galactan) but did not detect these compounds in our datasets.
631 Additional compounds that we identified remain to be explored (see Table S4).
632
633 **4.4. Disentangling dietary and environmental influences on T2D gut microbiomes**
634 We expect that our results showing altered potential metabolism for compounds detected within gut
635 metagenomes may arise from a combination of environmental exposures that supplement and shape gut
636 microbe assemblages, dietary inputs that further support or deplete members of the gut microbiome, gut
637 environment and function, and neurological influences, among other factors (Cryan et al., 2019).
638 Where we found matching trends across all case studies (e.g., arabinose, fructose, melibiose, BCFA-
639 ACPs, lignin), this may be consistent with an alignment between environmental exposures and dietary
640 inputs. In the scenario where compounds consistently showed in both T2D cohorts, but not in the
641 restoration gradient, this suggests dietary effects alone. For example, increased potential metabolism
642 results for amylopectin suggests that excessive consumption of starch-rich foods may contribute to
643 T2D. For many compounds we found alignment in potential metabolism between the ecosystem
644 condition and T2D gut (Chinese cohort), but not the T2D gut (Swedish cohort) – this may point to
645 greater environmental exposures and detrimental health influence in some population groups. Yet
646 another contrast occurred in the case of ammonia, where potential metabolism trends were opposing in
647 the T2D cohorts, in the absence of any ecosystem condition effect, which suggests a lone influence of
648 dietary exposures associating with T2D.
649

650 **4.5. Implications for community connection to soils and ecosystems**

651 Our findings suggest that filling knowledge gaps in the pathogenesis of T2D may require more holistic
652 consideration of linkages, especially at the systems level of health risk and promotion (consistent with
653 Handte-Reinecker & Sardeshpande, 2025); such as where landscape-scale ecosystem management may
654 drive changes in microbial functional capacities represented across assemblages of organisms. Our
655 findings suggest that maintaining balanced functioning healthy soil ecosystems in human habitats,
656 especially those used for food production, is increasingly important for supporting healthy human
657 populations. Increasing urbanization, ecosystem degradation, the industrialization of food production
658 and its distribution, and reducing time spent in nature have collectively reduced contact with soil
659 microbiomes for a large proportion of people. When humans are exposed to soil in modern
660 environments, it is increasingly within urbanized or highly modified landscapes (e.g., degraded
661 ecosystems with low natural biodiversity, highly modified land and disturbed soils, monocultural
662 agricultural fields) where microbiomes and their functional capacities have been greatly altered.

663 From a societal viewpoint, our findings suggest that addressing the global T2D pandemic may
664 benefit from restoring urban biodiversity, developing effective approaches for regenerative agriculture,
665 increasing soil biological health and diversity as key parts of a restorative continuum, and finding a
666 better balance between anthropogenic land use and nature. At the heart of this challenge lies the need
667 for a deeper understanding of soil health—recognizing soil as a complex living ecosystem in which the
668 microbiome plays a pivotal role. Understanding the critical links between soil health, food production,
669 and human health is fundamental for strengthening the diminished connections between people, land
670 and soil (Wall et al., 2015) and provides a foundation for developing strategies and actions that might
671 lead to improved gut health and resilience to T2D. This critical understanding of links between soil
672 ecosystem health and human health also draws on parallels of thinking about nature from an
673 Indigenous peoples' worldview. Indigenous knowledge offers guidance and support to theories that

674 place the health or condition of nature (land, biodiversity, water and air) as essential to maintaining
675 human health and wellbeing. Central concepts also recognize the importance of reconnecting with
676 nature by strengthening engagement with ecosystems through activities such as restoration and
677 regeneration. For example, in Aotearoa-New Zealand, Indigenous Māori stress connection and inter-
678 dependency with ecosystems through core principles such as ancestral links and association (Warbrick
679 et al., 2023). This includes concepts of whakapapa (genealogical descent of all living things) which
680 holistically reaffirms the importance of genetic and chemical assemblages in soils and landscapes to
681 support all life forms, including microbiomes (Harmsworth & Awatere, 2013). Concepts of
682 kaitiakitanga (guardianship of the environment) are also commonly used to reinforce actions and
683 strategies to guide environmental restoration and promote health. Indigenous perspectives offer
684 important guidance to all peoples for personally reconnecting and engaging with our ecosystems and
685 their stewardship in order to support healthy landscapes, soils, microbiomes and people.
686

687 **4.6. Study limitations**

688 There is well documented regional geographic variation in soil and gut microbiomes (Andreu-Sánchez
689 et al., 2025; Delgado-Baquerizo et al., 2018; Kumar & Bhadury, 2023; Vasar et al., 2022) which may
690 affect the interpretation of our results. We have examined a limited number of case studies to
691 demonstrate a proof-of-concept, because relevant case study metagenome datasets (with publicly
692 available metadata that span gradients of ecosystem quality while limiting uncontrolled sources of
693 variation) are rare. Although, we note that land uses and management that degrade ecosystems may be
694 driving homogenization and hence greater predictability of soil microbiome structure and functions in
695 urban areas (Delgado-Baquerizo et al., 2021; Sun et al., 2025). Our method aims to bypass variation
696 that is often inherent with taxonomically focused studies, via targeting the hypothetical potential
697 metabolism of compounds, based on the idea that microbiomes will be universally shaped by the

698 resources (e.g., feedstocks) and the environmental conditions available to them. It remains to be tested
699 whether the patterns we observed hold in other geographic regions and human populations, and
700 whether they may be replicated under controlled experimental conditions. The SUPER-FOCUS and
701 ModelSEED resources we used have not been actively updated in recent years and may be missing the
702 latest functional annotations. There may be some inaccuracies in the mapping from SUPER-FOCUS
703 functions to ModelSEED pathways and reactions, however we use rule-based scripts that can be
704 refined, consistent with other software development. We recognize this CPP method represents a
705 simplification of complex metabolic processes within microbiomes (e.g., it does not discern anabolism,
706 catabolism, reactants or products). Moving beyond this proof-of-concept, future work should use
707 updated resources that enable comprehensive linkage from functional pathways to reaction and
708 compound databases. Noting the conceptual difference in our approach (CPP reflects functional
709 potential imprinted in DNA), we suggest future work should compare CPP measures to quantitative
710 measurement of compounds present (e.g., via metabolomics). Control samples were not included in the
711 case study datasets analysed, however all samples are considered 'high biomass' with low risk of
712 contamination, and we removed human genome sequences (observed at low levels) from gut
713 metagenome samples.
714

715 **4.7. Conclusions**

716 We found overlapping trends in the potential metabolism of compounds with beneficial bioactivities
717 and involved in energy harvesting and management within separate model case study datasets of
718 degraded ecosystem soil from the United States and Australia, and T2D gut microbiomes from Swedish
719 and Chinese cohorts. Our results support existing studies implicating, at least in part, a role for
720 microbiomes in T2D. Additionally, our findings support the hypothesis that degraded soil microbiomes
721 may contribute to metabolic imbalances found in T2D via environmental microbial exposures and/or

722 nutrient-depleted (e.g., low mmBCFA content) food derived from them. Our novel method used to
723 examine microbiome potential metabolism at the resolution of individual compounds has derived new
724 insights into potential links between soil, ecosystem and T2D gut health that warrant further
725 transdisciplinary investigation.
726
727 **Data availability**
728 Code used to support this study is available from: https://github.com/liddic/cpp3d_t2d
729
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731 We acknowledge the authors of previously published metagenomics datasets used here, which enabled
732 comparison of samples spanning human and environmental health. We acknowledge Indigenous
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