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

Microbiomes connect ecosystems and humans under One Health. Yet, microbiome-mediated impacts of global change on human 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 change with ecosystem condition and may influence human metabolism through shaping food quality and gut microbiomes (e.g., functions, metabolites), including transfer of chemical compounds via microbial turnover. Here, we explored potential links between ecosystem degradation and metabolic alterations in T2D gut microbiomes by examining overlapping functional capacities in (1) soil microbiomes from ecosystem quality gradients (highly disturbed to mature/natural sites) and (2) gut microbiomes in T2D versus normal health. Often overlooked, metabolites are key components of microbiomes, with structural, functional and nutritional roles. Therefore, we sought to emphasize the compounds that might be accumulated, depleted and/or transferred via microbiomes, possibly independent of colonization. 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 potential metabolism of individual compounds. Examining selected compounds relevant to soil and gut health, and via exhaustive compound-wide trend analyses, we identified compounds relevant to energy harvesting and management 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 nutrient-depleted food and/or

52 excessive exposures associated with degraded ecosystem soil microbiomes may distort gut microbiome

53 functional capacities and metabolism and thereby contribute to type 2 diabetes.

54 **Introduction**

55 Environment, diet and lifestyle exposures under the global trends of urbanization, land use change,
56 ecosystem degradation, biodiversity loss and reduced nature contact are associated with many non-
57 communicable diseases, including several with suspected microbiome-mediated links (Flandroy et al.,
58 2018; Flies et al., 2019; Roslund et al., 2022). Among these, type 2 diabetes (T2D) is a major chronic
59 multifaceted metabolic disease with uncertain pathogenesis, characterized by insulin resistance, loss of
60 insulin secretion, high blood sugar levels and wider metabolic dysregulation (Beulens et al., 2022; Lu
61 et al., 2024; Pearson, 2019). Growing evidence supports the influence of gut microbiomes on human
62 metabolic health and dysfunction. However, there is a pressing need to better understand the
63 contribution of microbiomes in T2D (Cunningham et al., 2021; Gurung et al., 2020) due to their
64 potential mediating influence via diet, lifestyle and environmental factors. The role of environmental
65 microbiomes in supporting human metabolic health remains understudied (Flandroy et al., 2018)—
66 particularly in the context of accelerating global change (IPBES, 2018), and despite growing
67 recognition that microbiomes connect ecosystems and humans under One Health (Banerjee & van der
68 Heijden, 2022; Muhummed et al., 2025). T2D is a major contributor to the global non-communicable
69 disease pandemic (Cheema et al., 2014) and represents ~96% of total diabetes cases, which impacted
70 529 million people in 2021 (6.1% prevalence; costing US$966 billion), and is expected to rise to 1.31
71 billion people by 2045-2050 (10% prevalence; costing US$1.05 trillion) (Ong et al., 2023; Sun et al.,
72 2022).

73 The human gut microbiome is directly involved in our metabolism, shaping the biochemical
74 profile of our diet via an extensive metabolic repertoire that is distinct yet complementary to
75 mammalian digestive enzymes (Rowland et al., 2018). The gut microbiome and its diet-derived
76 metabolites influence human physiology and energy regulation by modulating appetite, energy harvest,
77 gene expression, gut motility, and the conversion and storage of dietary energy (Chao et al., 2025;

78 Krajmalnik-Brown et al., 2012). Specifically in T2D, shifts in gut microbiome community and
79 functional composition have been compared to normal healthy subjects, which are in part alleviated by
80 treatment with the antidiabetic drug metformin (Forslund et al., 2015). Moreover, fecal microbiome
81 transplantation studies have demonstrated amelioration of clinical indicators of T2D (Chen et al., 2023;
82 Wu et al., 2023). Long-term diet is a key factor shaping gut microbiome composition (Gilbert et al.,
83 2018), and diet shaped by the gut microbiome can modify the expression and operation of intestinal
84 enteroendocrine cells (EECs) responsible for sensing and responding to nutrients (Aliluev et al., 2021;
85 Ye et al., 2019). These are multidirectional interactions, as loss of EECs and their secreted hormones
86 also changes microbiome structures (Blot et al., 2023).

87 Intriguingly, numerous assessments attribute a major share of T2D risk to environmental
88 exposures that extend well beyond lifestyle and dietary behaviors to include the built, natural, social,
89 and physicochemical environments (Beulens et al., 2022). Among these, strong population-level
90 evidence links T2D to a shift in living environments from rural to rapidly growing, densely populated
91 urban areas (Attard et al., 2012; Cheema et al., 2014; Gassasse et al., 2017; Ruiz-Alejos et al., 2018).
92 Importantly, microbiomes may represent a key potential linkage mechanism between environmental
93 exposures consistent with T2D risk and metabolic processes occurring in the human gut. Gut
94 microbiomes are predominantly shaped by environmental factors, including diet and ambient
95 exposures, not host genetics (Rothschild et al., 2018).

96 In particular, soils represent the globally dominant source of environmental microbial
97 exposures, providing home to 59% to >99.9% of species on Earth (Anthony et al., 2023; Blakemore,
98 2025). The gut is dominated by spore-forming bacteria, together with facultative anaerobes, capable of
99 surviving and dispersing through aerobic environments before re-establishing in suitable oxygen-
100 deprived habitats (e.g., lower intestine, wet organic-containing soil habitats) (Browne et al., 2016;
101 Wyres & Holt, 2018). Butyrate-producing bacteria, which ferment organic matter to produce the short-

102 chain fatty acid butyrate and are considered key members of gut microbiota, are found widely in many
103 outdoor ecosystems and host organisms (Brame et al., 2024). Exposure to naturally biodiverse soil
104 microbiomes shapes and may supplement gut microbiome composition and functional capacity,
105 particularly from a young age (Liddicoat et al., 2020; Liu et al., 2021; Roslund et al., 2021; Wopereis et
106 al., 2014; Zhou et al., 2024). Gut microbiota variation in wild primates has been explained by
107 environmental heterogeneity, especially soil differences (Grieneisen et al., 2019). Consequently, soil
108 microbes and products of microbial activity and turnover (i.e., microbiome components) may be
109 introduced to the gut environment through direct soil contact, foodborne and airborne (e.g., dust)
110 microbiomes. Beyond the microbes per se, microbiomes also comprise important metabolites
111 (including constituent molecules, chemical compounds) and potentially transferable genetic material
112 (Peng et al., 2025) with diverse structural, functional and nutritional roles (Adamczyk, 2021; Bindels et
113 al., 2025; Daisley et al., 2021). Although the proportion of soil microbes that transfer and colonize the
114 gut is uncertain – the capacity for soil microbiomes to shape human gut microbiomes and influence
115 health and disease is well-recognized (Muhummed et al., 2025; Robinson & Breed, 2025; Roslund et
116 al., 2024).

117 Moreover, soil microbiome composition and functional capacity may shift in generalizable
118 ways with ecosystem quality (i.e., spanning anthropogenically disturbed/degraded to intact natural
119 condition), with potential to impact human health (Delgado-Baquerizo et al., 2021; Liddicoat et al.,
120 2024; Liddicoat et al., 2022). As urbanization accelerates—with around 70% of the global population
121 projected to live in cities by 2050 (UNDESA, 2019)—the degradation and homogenization of soil
122 microbiomes in urban and peri-urban environments may pose growing risks to human health (Sun et
123 al., 2023). Soil microbiomes are important for incorporating diverse nutrients into plant and animal-
124 derived foodstuffs – including via microbial nutrient gathering (Cao et al., 2024), direct incorporation
125 of metabolites and components of microbial bodies (Adamczyk, 2021), and influencing gut microbiota

126 of ruminants (Qin et al., 2022). Therefore, soil ecosystem degradation may also impact on human gut
127 microbiomes and metabolism through dietary pathways. Exploring overlaps in the functional capacities
128 of (1) degraded versus naturally biodiverse soil microbiomes (linked to aboveground degraded versus
129 intact ecosystem condition) and (2) T2D versus normal healthy gut microbiomes, may reveal
130 previously unappreciated microbiome linkages between environments and T2D.

131 Despite their importance, the measurement and definition of 'healthy microbiomes' in humans
132 and soils remain challenging (Fierer et al., 2021; Van Hul et al., 2024). Approaches that examine the
133 range of functions performed by microbiomes are expected to offer improved understanding over
134 measures based on taxonomic composition (Van Hul et al., 2024). Community-scale microbial
135 metabolism, where bioenergetic machinery and resources (including metabolites and electron transfer)
136 are shared in the extracellular space (Daisley et al., 2021), adds complexity and means that assemblages
137 may often combine to perform functional roles of interest. The need for improved insight into the
138 contributions of microbiomes in health and disease has motivated efforts to analyze soil and gut
139 microbiomes through the lens of their *compound processing potential* (CPP) (Liddicoat et al., 2024;
140 Figure S1). A compound-oriented approach can provide more direct functional information about
141 microbiomes because of known biochemical and health associations with chemical compounds (e.g.,
142 microbial feedstocks, substrates and metabolites, such as carbohydrates, lipids, proteins, amino-sugars,
143 vitamins), which are not described for most microbial taxa. New insights into differential compound
144 processing by microbiomes may also point to mechanistic hypotheses and/or possible therapeutic
145 targets. Microbial metagenomes (the entire collective DNA of a sample) are used to examine functions.
146 However, existing approaches require specialized knowledge of complex biochemical pathways and do
147 not provide resolution at the fundamental level of the chemical compounds involved (i.e., individual
148 compounds may often participate in multiple pathways). Existing metabolite prediction frameworks
149 (e.g., Garza et al., 2018; Mallick et al., 2019; Neveu et al., 2023) have their own strengths in particular

150 applications, but rely variously on metabolome training datasets, microorganism-specific genome-scale
151 metabolic models, taxonomic abundance estimates, and assumed environmental conditions, to model
152 short-term reaction rates. However, these approaches are unsuited to highly biodiverse, poorly
153 characterized and varying environments such as soils (Liddicoat et al., 2024). Thus, there is a need for
154 new, compound-oriented methods to examine normal versus aberrant functioning with a consistent
155 approach across soil and gut microbiomes.

156 Here we explore and compare model case study soil and gut metagenome datasets from
157 gradients of ecosystem quality and in subjects with T2D versus normal health by quantifying their
158 genomic potential to metabolize an array of chemical compounds. Specifically, the aims were to
159 improve functional and metabolic insights to the role of microbiomes in ecosystems and T2D, and their
160 potential overlaps, through identifying: (1) compounds with differential potential metabolism (i.e., with
161 trending CPP) based on analysis of soil metagenomes across an ecosystem quality gradient (i.e.,
162 degraded to intact natural condition); (2) compounds with differential potential metabolism (based on
163 CPP values) from gut metagenomes in untreated T2D versus normal health; and (3) overlapping
164 compounds and biochemical/functional pathways between (1) and (2) that may reveal new insights to
165 potential environmental microbiome contributions to T2D.

166

167 Materials and Methods

168 *Case study data*

169 We examined four model case study datasets (Table 1) comprising previously published (1) soil
170 metagenomes from a restoration gradient case study (United States, US; that compares degraded post-
171 mining, reforested, and unmined reference sites; Sun & Badgley, 2019) and disturbed versus natural
172 soils (as described in Liddicoat et al., 2024) from the Australian Microbiome Initiative (AMI; Bissett et
173 al., 2016); and (2) gut metagenomes from subjects with untreated T2D and normal healthy controls

174 (Forslund et al., 2015). We determined CPP trends with restoration (described below), however, our
175 subsequent focus was to characterize potential metabolism associated with degraded ecosystems (i.e.,
176 corresponding to the more disturbed soil microbiomes in the younger revegetation age samples of the
177 restoration gradient). We used two T2D datasets comprising separate case study cohorts. The first T2D
178 dataset from Sweden (Karlsson et al., 2013) was analyzed to identify differences in CPP or potential
179 metabolism in T2D, and then assess overlaps with trending CPP found in more degraded (towards
180 younger) samples from the restoration gradient (US) case study. A second T2D dataset from China
181 (i.e., '1st validation dataset') (Qin et al., 2012) was then integrated to confirm and consolidate CPP
182 trends that emerged consistently across the restoration gradient and both T2D case studies. Lastly,
183 corroboration of overlapping CPP trends was sought in the disturbed versus natural AMI soils dataset
184 (i.e., '2nd validation dataset').
185
186 **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**
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)[a]; Illumina HiSeq	Sweden

T2D vs. Normal (1st validation dataset)	T2D met- (n = 30; females *n* = 17, aged 51-71 years; males *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	Functions (SUPER-FOCUS): *n* = 19,352 (8650 ± 4214). Functional % all sum to 100%. Compounds (CPP profiling): *n* = 7,213 (5,310 ± 1670). CPP (%): (65.7 ± 4.1 %)	SRA accession PRJNA422434 (Forslund et al., 2015; Qin et al., 2012) [a]; Illumina Genome Analyzer II	China
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) (2nd 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) [b]; Illumina HiSeq	Australia

Note: [a] 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, we only included subjects >50 years old to compare with the mature age (69-72 years) Swedish subjects. [b]AMI Data portal URL: https://data.bioplatforms.com/organization/australian-microbiome; AMI soils were surface

193 depth (0–10 cm), from the temperate climate zone, with 7.5–45 % clay content (i.e., avoiding very
194 sandy and very clayey soils).
195
196 ***Metagenome functional profiling via compound processing potential (CPP)***
197 Our CPP analysis approach uses a novel bioinformatics workflow that redistributes values of functional
198 potential relative abundances (%), from the scale of functions (i.e., biochemical pathways that often
199 comprise multiple linked chemical reactions), down to the level of the individual chemical compounds.
200 These compounds are conceptually participating in the array of intersecting chemical reactions
201 represented in the collective functional capacity of a metagenome. These methods do not measure
202 actual chemical compounds. Rather, they quantify the genetic capacity of a microbiome to metabolize
203 (or process) compounds, as written in the metagenome. The distinction is not made between
204 compounds being consumed (reactants) or produced (products) as functional pathways may often
205 involve strings of chemical reactions (metabolic processes), involving one or many organisms, where a
206 compound can be the output of one reaction and an input to another.
207 Measures of CPP were derived following the steps outlined in Figure 1. Firstly, following
208 Liddicoat et al. (2024), using high performance computing resources (Flinders_University, 2021;
209 Pawsey_Supercomputing_Research_Centre, 2023) raw metagenomic sequences were accessed,
210 trimmed for quality control, and human case study sequences that mapped (using Bowtie2; Langmead
211 & Salzberg, 2012) to the human genome (GRCh38.p14 / hg38) were excluded (low levels were
212 observed; Supplementary Material, Supporting Information). Then good quality read 1 sequences were
213 classified to SEED subsystem (Overbeek et al., 2013) functional annotations and relative abundances
214 using SUPER-FOCUS software (Silva et al., 2015). Every SUPER-FOCUS function (output row) was
215 translated to one or more corresponding chemical reaction(s) using a purpose-built R-script algorithm
216 based on ModelSEED database lookup tables (from

217 https://github.com/ModelSEED/ModelSEEDDatabase; accessed 10 Aug 2022). Linking of functions
218 (and sub-functions where present) to chemical reactions was based on either: full matching of
219 functional hierarchies (using subsystem-class, -subclass, -name and -role); detection of Enzyme
220 Commission (EC) number; or matching of SUPER-FOCUS function name within ModelSEED lookup
221 tables for reactions (reaction name or alias), subsystems (role), or reaction-pathways (external reaction
222 name). Reactions were then linked to corresponding compounds using ModelSEED database tables
223 ('reactions.tsv', 'compounds.tsv'). Advancing from the previous method used in Liddicoat et al.
224 (2024), here we derived CPP measures at the resolution of individual compounds (all software
225 parameters and supporting R code for CPP calculations are available at:
226 https://github.com/liddic/cpp3d_t2d):

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

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

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

240

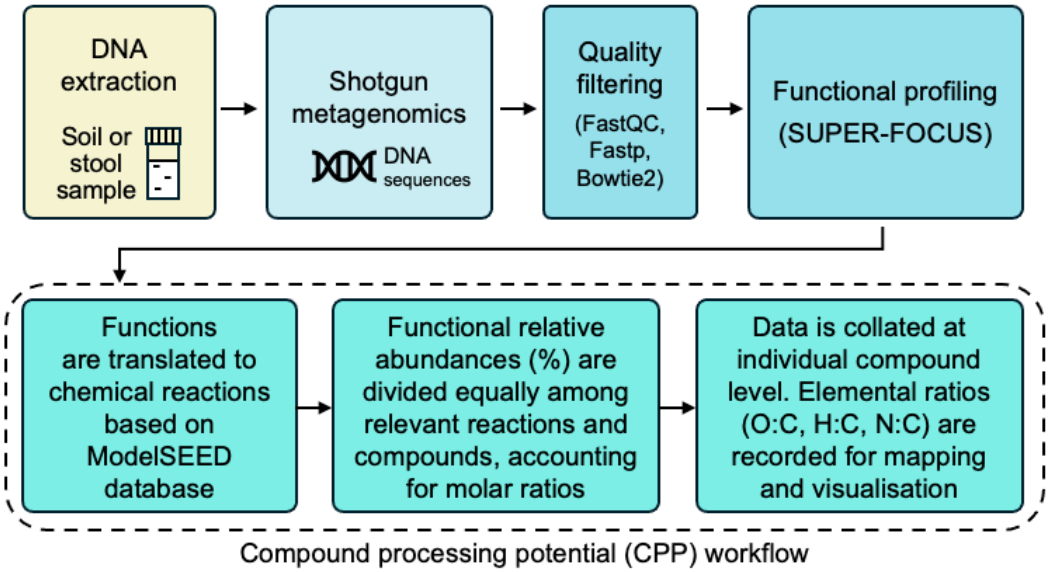

Figure 1. Overview of the CPP analysis workflow used to translate microbial metagenome functional potential relative abundances to quantify potential metabolism at the level of individual compounds.

Statistical Analysis

We used a staged approach to explore overlapping potential metabolism (i.e., CPP) trends in the soil and T2D gut samples. We first examined the restoration gradient soils (US), then progressively considered the T2D (Swedish cohort, SWE) and T2D (Chinese cohort, CHN) datasets, and lastly the AMI soils to corroborate findings (further detail is in Supplementary Material and at https://github.com/liddic/cpp3d_t2d):

a. ***Thirty selected compounds*** were chosen due to their relevance to soil and/or gut health (e.g., Blanco-Pérez et al., 2021; Daisley et al., 2021; Krajmalnik-Brown et al., 2012; Six et al., 2006; compounds and reference information are listed in Table S1). For this list of compounds, CPP trends were evaluated using Kendall's tau rank correlation tests based on ordinal revegetation age classes. We interpreted CPP trends with ecosystem degradation as the inverse of calculated trends with increasing revegetation age (towards intact natural condition). This assumption that degradation may be modelled as the opposite of restoration is based on previous work showing

258 highly disturbed post-mining soil microbiomes progressively shift in their structure with
259 ecosystem restoration (Liddicoat et al., 2022). CPP trends (i.e., differences) with T2D versus
260 normal health were evaluated using non-parametric Wilcoxon rank-sum (Mann-Whitney) tests,
261 due to often differing variance of groups (T2D vs. normal) and to avoid normality assumptions.
262 All data were plotted to assist interpretation.

263 b. ***Compound-wide association tests*** were also performed to exhaustively scan all compounds
264 represented in the respective CPP datasets, using the same trend evaluation methods as above.
265 In soils, compounds that did not have relative abundance data for at least a quarter of the total
266 sample number were excluded due to low data. In restoration gradient soils, strong trends were
267 discovered, and only significant results based on adjusted *P*-values were considered further (i.e.,
268 after applying Benjamini and Hochberg false discovery rate correction). In the separate
269 compound-wide analysis of T2D gut samples, compounds were only included if at least one or
270 other of the T2D or normal groups contained at least 50% non-zero CPP values. Testing was
271 based on $\log_{10}$-transformed CPP % values, after zero replacement with small positive values
272 equal to half the minimum non-zero CPP % of all samples. One-sided (directional) difference
273 tests were based on whether the median value in T2D was greater or less than the median value
274 for normal healthy subjects. The T2D (SWE) Wilcoxon rank sum test results yielded many *P*-
275 values ≤0.05. However, none were low enough to withstand Benjamini and Hochberg *P*-
276 adjustment for multiple testing, therefore, *P*-adjustment was not performed in the compound-
277 wide T2D gut data to retain the most interesting (potentially significant) results for further
278 exploration.

279 c. ***Overlap analysis.*** Compounds with CPP % trends in both the restoration gradient (US) and
280 T2D (SWE) case studies were identified, based on matching ModelSEED compound identifiers.

281 Our focus was to highlight compounds that displayed consistent increases or decreases in CPP
282 % with degraded/disturbed ecosystems (i.e., towards younger revegetation age) and in T2D.
283 d. ***Visualising compound-wide association test results.*** Next, compounds with CPP % trends in
284 the restoration gradient (US) and T2D (SWE) gut microbiomes, and overlaps between the two,
285 were mapped using their chemical formula elemental ratios of O:C, H:C, and N:C. From this
286 mapping space, prominent localized clusters of consistently trending compounds (i.e., with
287 ecosystem degradation and T2D) were identified and further analyzed to determine trends in
288 their collective (summed) CPP % and to interpret their possible metabolic importance.
289 e. ***Trend confirmation and consolidation.*** The above steps were repeated with the second T2D
290 (CHN) cohort or '1st validation dataset', which refined the list of individual compounds with
291 parallel trends in CPP values across the restoration gradient (US) and both T2D datasets. Trend
292 and contextual information for these compounds was reported and visualized to represent the
293 significance ($-\log_{10}$[*P*-value]; as used in genome-wide association study Manhattan plots) of
294 their association with disturbed/degraded ecosystems and T2D, and the number of functional
295 themes (level 3 subsystems) they are linked to. Here, data for T2D were visualized using mean
296 results from the Swedish and Chinese cohort datasets. To corroborate parallel CPP trends from
297 the restoration gradient (US) and T2D (SWE, CHN) datasets, a focused analysis of CPP trends
298 only for previously identified compounds was performed in disturbed versus natural AMI soils
299 (i.e., '2nd validation dataset').
300 f. ***Beta diversity ordinations.*** Supplementary analyses using the restoration gradient and Swedish
301 cohort dataset were performed to examine community-scale compositional patterns and
302 differences between sample groups and analysis approaches, comparing conventional functional
303 potential (%) data (termed 'functions') versus CPP (%) data. This analysis used principal
304 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.

Results

Potential metabolism trends with ecosystem degradation

In the restoration gradient (US) soils, 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.

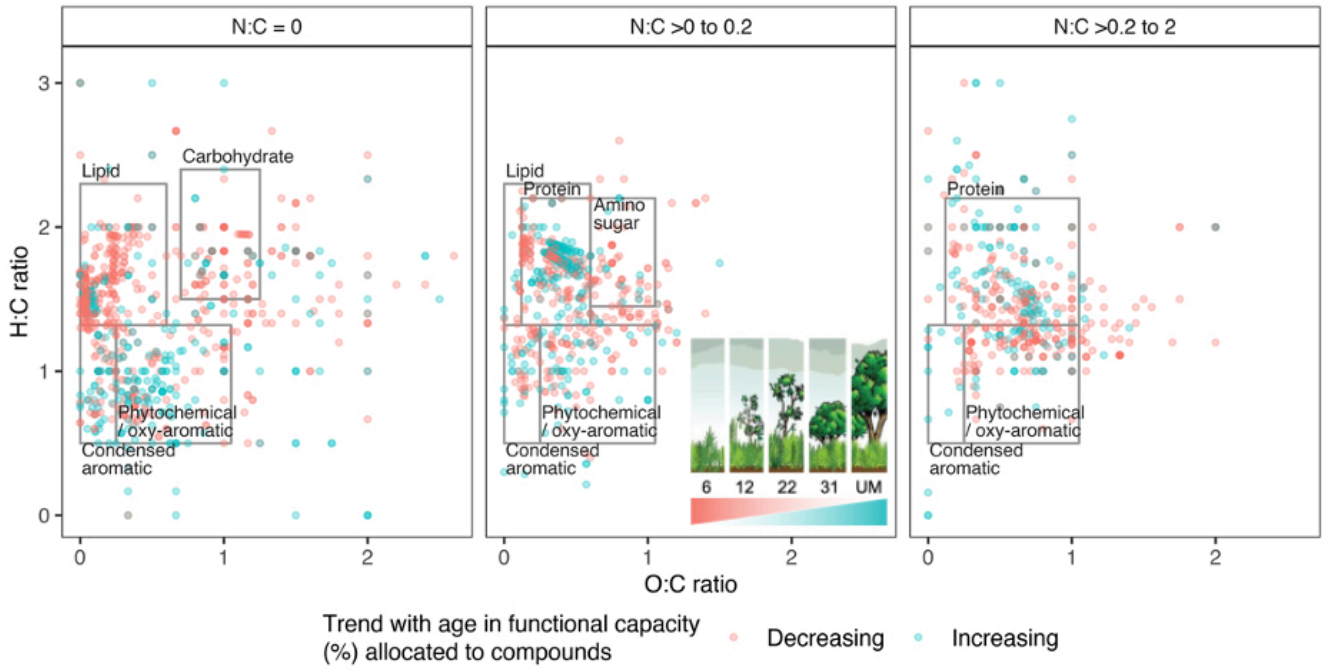

Figure 2. Compound-wide trend analysis results derived from restoration gradient (US) soil metagenomes (described in Table 1), showing carbon-containing compounds with increasing (aqua, *n* = 720) or decreasing (red, *n* = 1,255) compound processing potential (CPP, i.e., functional capacity % or potential 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 metabolism (i.e., shifting CPP % values) from degraded to mature/natural ecosystem quality are displayed. Clusters of points indicate energetically and chemically similar compounds. The O:C by H:C panels are zoomed in from their full extent to improve visualization of more data-rich regions. N:C ratios are displayed as three depth-slices to represent the third dimension. Rectangular zones denoting compound classes are provided as a visual guide only (adapted from Rivas-Ubach et al., 2018; Wu et al., 2018).

343

344 ***Potential metabolism trends in T2D***

345 In the 30 selected compounds, we found consistent trends in the Swedish (SWE) and Chinese (CHN)
346 cohort datasets (Table S1; Figures S3-S32, S34) for increased potential metabolism in T2D for fructose
347 (SWE: $P \leq 0.05$, CHN: $P < 0.01$), arabinose (SWE: $P \leq 0.05$, CHN: $P < 0.001$), and amylopectin (SWE
348 and CHN: $P \leq 0.05$). We found decreased potential metabolism in T2D for lignin (SWE and CHN: $P \leq$
349 0.05).

350 Numerous trends were only found in one T2D cohort or the other. Increased potential
351 metabolism was found in T2D in the Swedish cohort for sucrose and starch ($P \leq 0.05$), and in the
352 Chinese cohort for mannose ($P < 0.001$); glucose, galactose and xylose (all $P < 0.01$). Decreased
353 potential metabolism was found in T2D in the Chinese cohort for acetate, propionate, butyrate, carbon
354 dioxide, hydrogen sulfide, indole (all $P < 0.001$); Menaquinone (Vitamin K2), glucosamine ($P < 0.01$);
355 methane, p-cresol and serotonin (all $P \leq 0.05$). We found conflicting potential metabolism trends for
356 ammonia across the cohorts, which increased in Swedish ($P < 0.01$) but decreased in Chinese ($P <$
357 0.001).

358 The compound-wide Wilcoxon rank sum trend analyses in T2D Swedish and Chinese cohorts
359 identified similar numbers of compounds with increased potential metabolism, but substantially higher
360 numbers of compounds with decreased potential metabolism in the Chinese cohort (visualized in
361 Figures S35 and S36; compounds and trend data for both T2D datasets are available in Supplementary
362 Material). For consistency, across both compound-wide T2D association analyses, and to retain the
363 most interesting T2D (SWE) cohort data, these data were not subject to *P*-adjustment for multiple
364 testing. In the T2D (SWE) data, a total of 1269 compounds with trending potential metabolism (n =
365 911 decreased, $n = 358$ increased) were found. From these, a total of 1181 carbon-containing
366 compounds ($n = 860$ decreased, $n = 321$ increased) were mapped using chemical formula ratios O:C,

H:C and N:C (Figure S35). For the T2D (CHN) data a total of 4292 compounds (*n* = 3984 decreased, *n* = 308 increased) were identified, corresponding to 3964 carbon-containing compounds (*n* = 3695 decreased, *n* = 269 increased) mapped using chemical formula ratios O:C, H:C and N:C (Figure S36). From visual inspection (comparing Figures S35 and S36), the chemical ratio mapping displays some zones of similarity (e.g., in the vicinity of carbohydrates) but also dramatic differences due to the widespread number of compounds with reduced potential metabolism in the T2D (CHN) dataset.

Consistent CPP trends with ecosystem degradation and T2D

Within the list of 30 selected compounds (Table S1), we found limited cases of consistent potential metabolism trends across all three datasets – i.e. with ecosystem degradation (US), T2D (SWE), and T2D (CHN). Specifically, we found increased potential metabolism in fructose and arabinose and decreased potential metabolism in lignin was consistent across all datasets (Table S1).

Using the compound-wide trend results from T2D (SWE) and restoration gradient (US) datasets, we performed an overlap analysis, based on matching ModelSEED compound identifiers, to highlight compounds with potential metabolism trends across both case study scenarios (compound and trend data are in the Supplementary Material). From a total of 304 compounds that had CPP trends with T2D (SWE) and across the restoration gradient, 286 carbon-containing compounds were visualized through chemical ratio mapping (Figure 3). Decreased CPP (i.e., potential metabolism) with T2D (SWE) and ecosystem degradation was observed in 70 compounds (66 contained carbon; red dots in Figure 3). Increased CPP with T2D (SWE) and ecosystem degradation was observed in 89 compounds (81 with carbon; green dots in Figure 3). Matching trends with ecosystem restoration were also found. Decreased CPP with T2D (SWE) and ecosystem restoration was observed in 95 compounds (90 contained carbon; orange dots in Figure 3). Increased CPP with T2D (SWE) and ecosystem restoration was observed in 50 compounds (49 with carbon; blue dots in Figure 3).

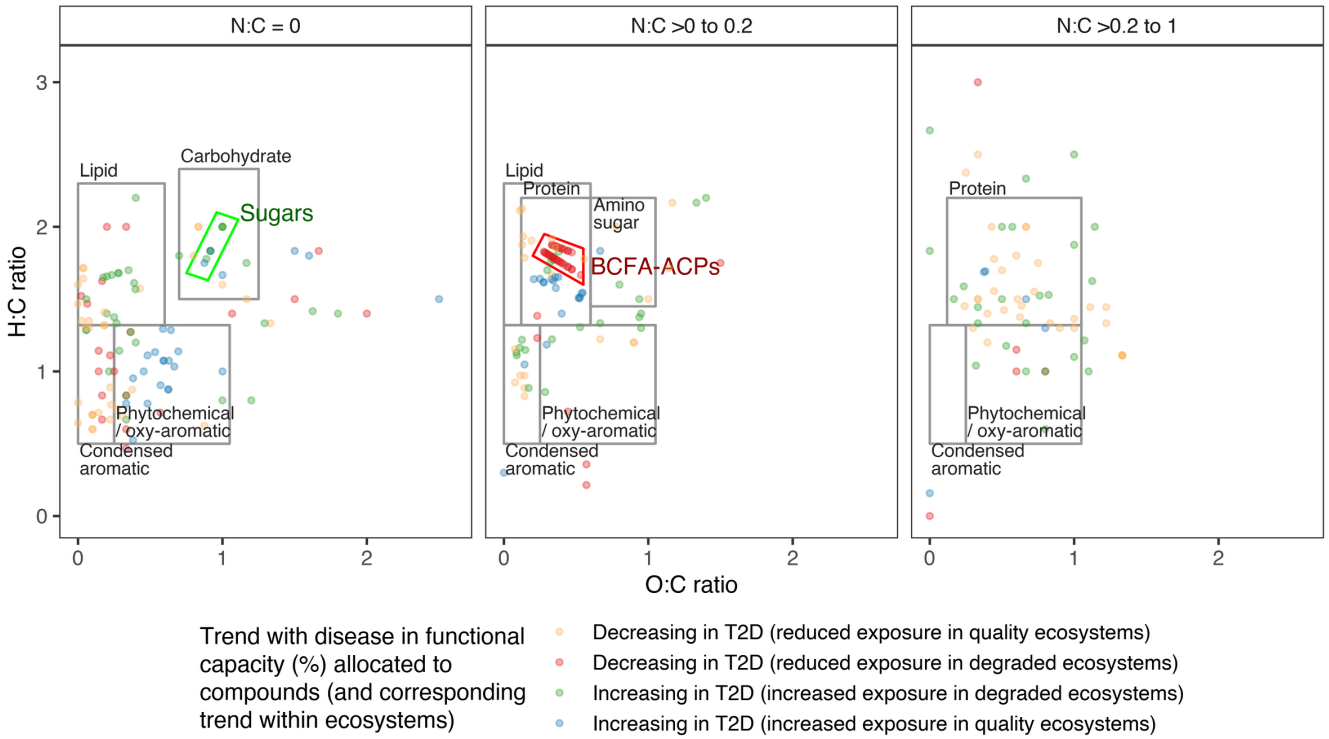

Figure 3. Overlap analysis showing carbon-containing compounds with trending potential metabolism in both compound-wide trend analyses of the T2D (Swedish cohort) and restoration gradient (US) case study datasets (i.e., this plot shows overlapping compounds from Figures 2 and S35). Clusters of each color represent energetically and chemically similar compounds occurring in each of the four trend groups listed (numbers of compounds are provided in the text). Further description of the plot format is provided in Figure 2. Expanded views of the grouped 'sugars' and branched-chain fatty acid - acyl-carrier proteins ('BCFA-ACPs') are provided in Figures S37-S41.

With our focus on microbiome functional shifts with ecosystem degradation, and to further explore compounds that also shared potential metabolism trends with T2D (SWE), we inspected the mapping visualization in Figure 3 and observed two prominent clusters of compounds with matching trends (also see Table S2-S3, Figures S37 and S39). The first group outlined in green and labelled

405 ‘Sugars’ in Figure 3 comprised six carbohydrate compounds (L-arabinose, melibiose, manninotriose,
406 D-lyxulose, sucrose, D-fructose; Table S2) that all display increasing CPP in both T2D (SWE) and
407 with ecosystem degradation (US). In the O:C, H:C, N:C mapping space, these sugars all occur on the
408 line H:C = 2 × O:C, with N:C = 0 (i.e., green points in Figure S37). Analysis of summed CPP values
409 for these sugars showed that they collectively exhibit a very strong increase with ecosystem
410 degradation (Kendall’s tau ~restoration = -0.844, P = <0.001) and a strong increase in T2D (SWE)
411 (Wilcoxon rank sum W = 985, P = 0.002) (Figures 4 and S38).
412 The second group outlined in red in Figure 3 comprised 35 unique compounds involved in fatty
413 acid biosynthesis. This group was labelled BCFA-ACPs (branched-chain fatty acid – acyl-carrier
414 proteins) as it is dominated by this type of compound (n = 30), together with n = 5 straight-chain fatty
415 acid-ACPs (Table S3). All compounds in the BCFA-ACPs group have reduced CPP in T2D (SWE) and
416 reduced CPP in degraded ecosystems, and they each occur on two straight lines in the O:C, H:C, N:C
417 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
418 the range >0 to 0.2; Figure S39). Analysis of summed CPP values for the BCFA-ACPs group showed
419 that they collectively exhibited a very strong decrease with ecosystem degradation (Kendall’s tau
420 ~restoration = 0.741, P = <0.001) and also moderate reduction in T2D (SWE) (Wilcoxon rank sum W
421 = 518, P = 0.023) (Figures 4 and S40). When the summed CPP data for these groups were validated in
422 the T2D Chinese cohort data, they showed very strong agreement (sugars: Wilcoxon rank sum W =
423 1149, P <0.001, BCFA-ACPs: W = 335, P <0.001; Figure 4, detailed plots in Figure S41).
424

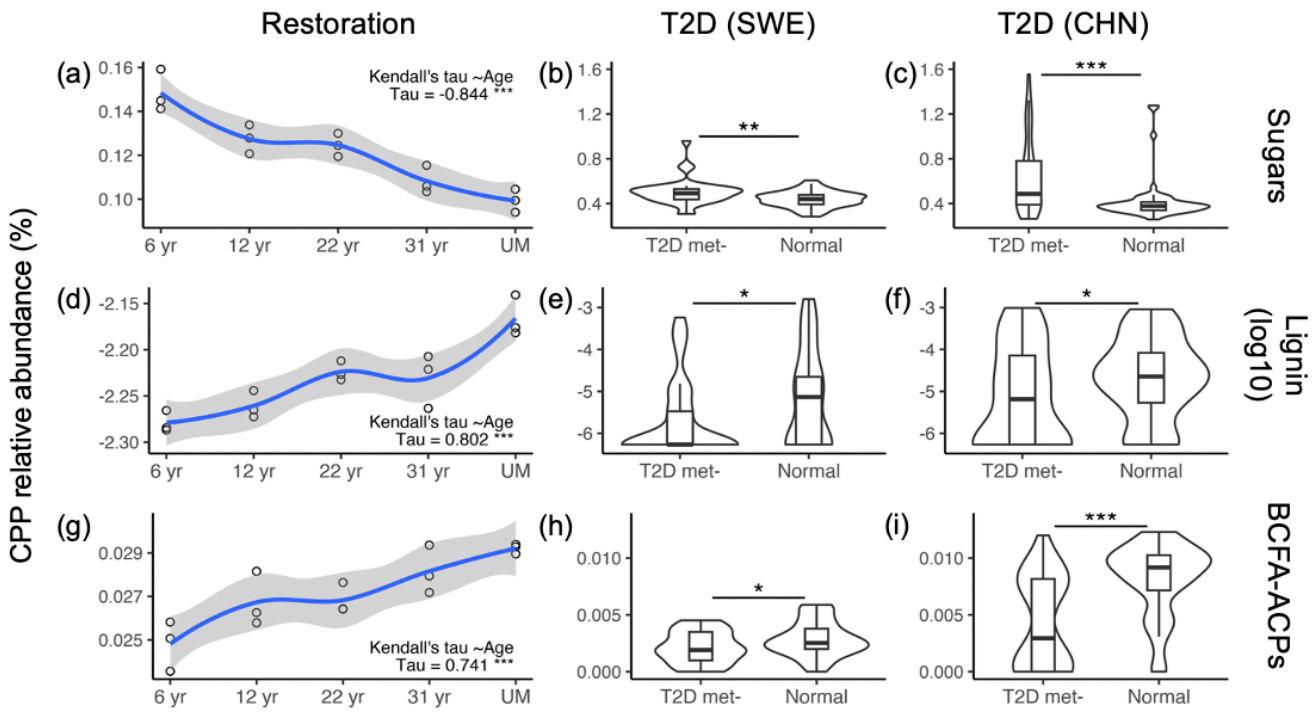

Figure 4. Potential metabolism of sugars (a-c), lignin (d-f), and branched-chain fatty acid – acyl carrier proteins (BCFA-ACPs, listed in Tables S3, S4) (g-i), within the restoration gradient (US; spanning restoration planting ages 6, 12, 22, 31 years and unmined, UM) (a, d, g), T2D (Swedish cohort, 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.

To confirm and consolidate individual compound trends exhaustively across all compound-wide trend results, we further isolated only those ModelSEED compound identifiers with matching trends across the restoration gradient (US) and T2D (SWE, CHN) case studies. This process identified 76

440 compounds with *n* = 60 decreased, *n* = 16 increased with ecosystem degradation (US) and both T2D
441 datasets (Figure 5; Table S4; compound and trend data are further detailed in Supplementary Material).
442 Summary trend and contextual information are displayed in Figure 5, using mean results from T2D
443 (SWE) and T2D (CHN) cohort datasets. From this filtering process at the individual compound level,
444 remarkably, the signal of decreased potential metabolism for all 35 compounds in the BCFA-ACPs
445 group previously identified from overlapping restoration gradient and T2D (SWE) datasets also
446 remained when intersected with the T2D (CHN) dataset.

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

458 As monomethyl BCFA-ACPs dominated this trend group, we were curious to understand
459 whether they might feature there exclusively, or if similar compounds were also present throughout the
460 data and not display trends. We found hundreds of other unique non-trending ACP-containing
461 compounds (*n* = 296 restoration, *n* = 289 T2D-SWE, *n* = 293 T2D-CHN), spanning a multitude of
462 enzymes or sub-functions (*n* = 223 restoration, *n* = 137 T2D-SWE, *n* = 146 T2D-CHN). Focusing only
463 on the methyl-containing variants, a shared inventory of non-trending ACP compounds was found

464 across all three datasets. Intriguingly, there were no other monomethyl unsaturated (-enoyl) BCFA in
465 the datasets beyond those that featured in our BCFA-ACPs trend group. The remaining shared non-
466 trending methyl-containing ACP compounds (*n* = 56) either contained carbonyl (-oxo-) groups (*n* =
467 18), or hydroxyl (-hydroxy-) groups (*n* = 22), or ester groups (*n* = 9), while the remaining (*n* = 7) were
468 saturated BCFA-ACPs (one short chain, and six long 13-16 chain length). These latter, non-trending
469 saturated BCFA-ACPs also differed from the saturated BCFA-ACPs in our trend group, which
470 contained mostly medium-length chains (Table S3).

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

476 Lastly, corroboration of the majority of observed overlapping trends was found in the
477 Australian soil metagenomes ('2nd validation dataset'). In disturbed AMI soils, we observed increased
478 potential metabolism of L-Arabinose, D-Fructose, Melibiose and 6-Phosphosucrose, and decreased
479 potential metabolism of lignin and all 35 of the BCFA-ACPs (all $P = <0.001$; Figure S42). From the 76
480 compounds with matching potential metabolism trends in degraded soils (restoration gradient, US),
481 T2D (SWE) and T2D (CHN), a total of 65 compounds (85.5 %) displayed shared potential metabolism
482 trends in the AMI disturbed soils (Table S4).
483

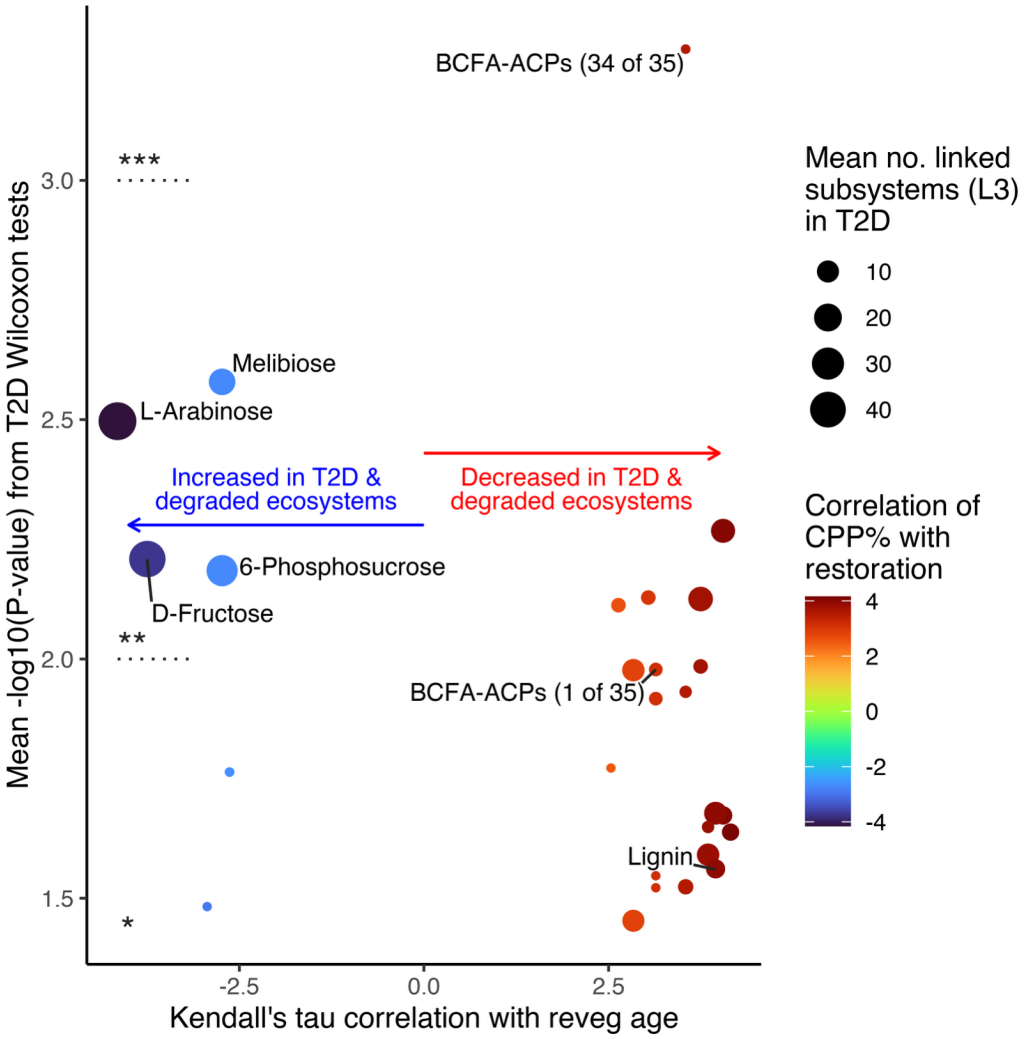

Figure 5. Relative importance of compounds with consistent trends across restoration gradient (US) and T2D (Swedish and Chinese) case study data, showing increased or decreased compound processing potential (CPP %, i.e., potential metabolism) with ecosystem degradation (soil) and in T2D (gut) microbiomes. 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)

493 subsystems (functional themes) linked to each compound, are calculated as mean values from the
494 Swedish and Chinese cohorts. Further details for compounds (n = 76 total) are in Table S4.
495

496 **Discussion**

497 We identify several meaningful overlapping trends in the functional capacity of soil and human gut
498 microbiomes, supporting our new hypothesis that nutrient-depleted food (e.g., low BCFA and lignin
499 content, high sugar content) and/or excessive microbiome exposures associated with degraded
500 ecosystem soil may contribute to the development of type 2 diabetes. These linkages warrant further
501 investigation due to the rising prevalence and impact of T2D and the widespread and increasing
502 processes of global change impacting soil microbiomes – which underpin food quality and provide a
503 key microbial reservoir for shaping the human gut microbiome. A possible alternative or
504 complementary explanation may be that similar ecological stresses and dysfunction in soil and gut
505 ecosystems may be driving convergence in microbial functional traits.

506 Using a novel method, we translated community-scale microbial metagenome functional
507 capacities to quantify potential metabolism for individual compounds. This compound-oriented
508 approach facilitates linkages to a wealth of published literature that links chemical compounds with
509 metabolism, health and wider biological processes in humans and the environment. Our mapping of
510 compounds into the bioenergetically informed O:C, H:C and N:C coordinate space helped to reveal
511 meaningful patterns of simultaneously trending compounds (e.g., sugars, BCFA-ACPs) with soil-
512 ecosystem quality and T2D versus normal health.
513

514 ***Do branched-chain fatty acids connect healthy ecosystems and healthy people?***

515 Our data suggest that microbiomes in degraded ecosystems and T2D have reduced capacity for
516 monomethyl BCFA (mmBCFA) biosynthesis. This may reflect a shift in the biochemical composition

517 of bacterial assemblages as mmBCFAs are key components that enhance bacterial membrane fluidity,
518 and mmBCFA synthesis capacity enables membrane fluidity-related adaptive responses for survival
519 and proliferation through environmental stresses (Gozdzik et al., 2023; Yoon et al., 2015). BCFA levels
520 vary among bacterial taxa, being commonly higher in Gram-positive bacteria, present in some Gram-
521 negative species, and especially high in *Bacillus* and *Bifidobacterium* (Yang et al., 2025; Yoon et al.,
522 2015). Within ecosystems, BCFAs are present in lipid/waxy plant compounds, animal (including
523 human) tissues and fluids, including ruminant products (milk, cheese, meat), fish, organs, blood serum,
524 skin, hair, adipose tissue, colostrum, breast milk, *vernix caseosa* of newborns, and at very high levels in
525 saprotrophic fungi such as *Conidiobolus* spp. found in soil and decaying organic matter (Gozdzik et al.,
526 2023; Maheshwari et al., 2020; Mao et al., 2023). Therefore, variation in BCFA synthesis capacity
527 could result from changes in microbial taxonomic composition as ecosystems degrade.
528 Monomethyl BCFAs are of interest because they possess multiple beneficial bioactivities for
529 human health and provide a direct link between bacterial and human physiology (Mao et al., 2023).
530 Beneficial bioactivities of mmBCFA include lipid-lowering, reducing the risk of metabolic disorders,
531 maintaining normal β cell (i.e., insulin-producing) function and insulin sensitivity, anti-inflammation,
532 cytotoxicity to cancer cells and regulation of development (Gozdzik et al., 2023; Mao et al., 2023;
533 Yang et al., 2025). Bacterially-derived mmBCFAs facilitate the direct biochemical crosstalk (i.e.,
534 uptake and cross-domain metabolism) between the gut microbiome and animal hosts (Brown et al.,
535 2023; Zhu et al., 2013; Zhu et al., 2021). In the nematode *Caenorhabditis elegans* and mammalian
536 tissue culture cells, mmBCFA have been shown to be critical for triggering the host mechanistic target
537 of rapamycin complex 1 (mTORC1) (Zhu et al., 2013; Zhu et al., 2021), a central regulator of cell
538 growth and metabolism (Takahara et al., 2020). The host mTORC1 responds to environmental signals
539 (e.g., amino acid availability, growth factors, energy levels, stress) to coordinate cellular status.
540 However, mTORC1 dysregulation is closely linked to diabetes, cancer, and neurodegenerative

541 disorders (Takahara et al., 2020). Liu et al. (2017) reported high capacities for human fetal intestinal
542 epithelial cells (enterocytes) to incorporate long-chain BCFA into membrane phospholipids. Following
543 uptake by human intestinal epithelial cells, further potential interactions between BCFA and host
544 endogenous BCFA synthesis and metabolism may occur (Gozdzik et al., 2023; Mao et al., 2023),
545 including post-translational modifications to cell proteins, which alter intestinal cell function (Ezzine et
546 al., 2022). While the precise mechanisms are uncertain, it is plausible that mmBCFA in the gut
547 microbiome might modulate host cellular metabolism and physiological functioning via these
548 interactions.

549 Microbial sources play a dominant role in the supply of health-associated mmBCFA (Mao et
550 al., 2023; Yang et al., 2025). Key inputs to BCFA synthesis include branched-chain amino acids (which
551 can only be made in bacteria, plants, and fungi, but not in animals; Neinast et al., 2019) or shorter
552 BCFA, which can be elongated. Microbes (e.g., rumen bacteria) also contribute to BCFA synthesis in
553 animals (Mao et al., 2023). Subsequently, both diet and gut microbiome synthesis provide major
554 sources of BCFA to humans (Mao et al., 2023; Yang et al., 2025). Within microbial environments (e.g.,
555 in soil or the gut), the interchange of BCFA between substrates and microbes can be expected because
556 fatty acids are a high-value energy source, and bacteria can assimilate environmental BCFA into their
557 bodies (Sen et al., 2016; Yao & Rock, 2015). In the gut, the constant release of a portion of mmBCFAs
558 from bacterial cell membranes will occur due to the rapid turnover of bacterial cells from competitive
559 interactions (Booth et al., 2023) and the secretion of fatty acids and their derivatives (Brown et al.,
560 2023). A portion of these gut bacteria may be supplied from external ecosystem exposures (Roslund et
561 al., 2021; Rothschild et al., 2018), while BCFA-synthesizing bacteria may be further shaped by diet and
562 other factors (e.g., high BCFA-content Bifidobacterium spp. proliferate in infants receiving breast milk;
563 Stewart et al., 2018).

As ecosystems degrade, there may be several reasons why BCFA-synthesizing organisms decline. Ecosystem degradation is widely associated with reductions in soil organic matter (e.g., Chen et al., 2018; Cunningham et al., 2015; IPBES, 2018; Kooch et al., 2022). Indeed, the more degraded soils from the restoration gradient case study dataset had lower soil organic content than unmined sites (Avera et al., 2015). Soil organic matter comprises a continuum of progressively decomposed organic compounds originating from plant residues, root exudates, microbial synthesis, and other chemical reactions, and commonly includes amino acids and fatty acids (Song et al., 2024). Amino acids can be released from proteins in the soil through proteolysis at rates correlated with soil organic matter pools and soil protein concentrations (Näsholm et al., 2009). This release increases with ecosystem succession (Kielland et al., 2007). Natural decomposition and recycling networks of saprotrophic fungi (which includes many high BCFA-content spp.) may also be degraded, likely reducing BCFA content and turnover in soil microbiomes. Diverse plant litter contributes to increased soil carbon, higher microbial abundance, diversity, decomposition and turnover, which strengthens over time (Zhang et al., 2023). In contrast, urban areas exhibit dramatically reduced fungal abundance and diversity compared to natural ecosystems (Abrego et al., 2020). Urbanization has been associated with the homogenization of soil microbiomes (Delgado-Baquerizo et al., 2021), which in turn reduces the range of assemblages available to shape and possibly resupply the human gut microbiome. Taken together, these influences may reduce access to and recycling of both branched-chain amino acids and fatty acids for soil microbiomes in degraded ecosystems, thereby potentially reducing ambient human exposure to health-promoting microbial mmBCFA synthesis capacity (Figure 6). Degraded agricultural soils might also produce crops and ruminant products (e.g., meat, dairy) with reduced mmBCFA content, that impact gut microbiomes via diet. However, these ideas require further investigation.

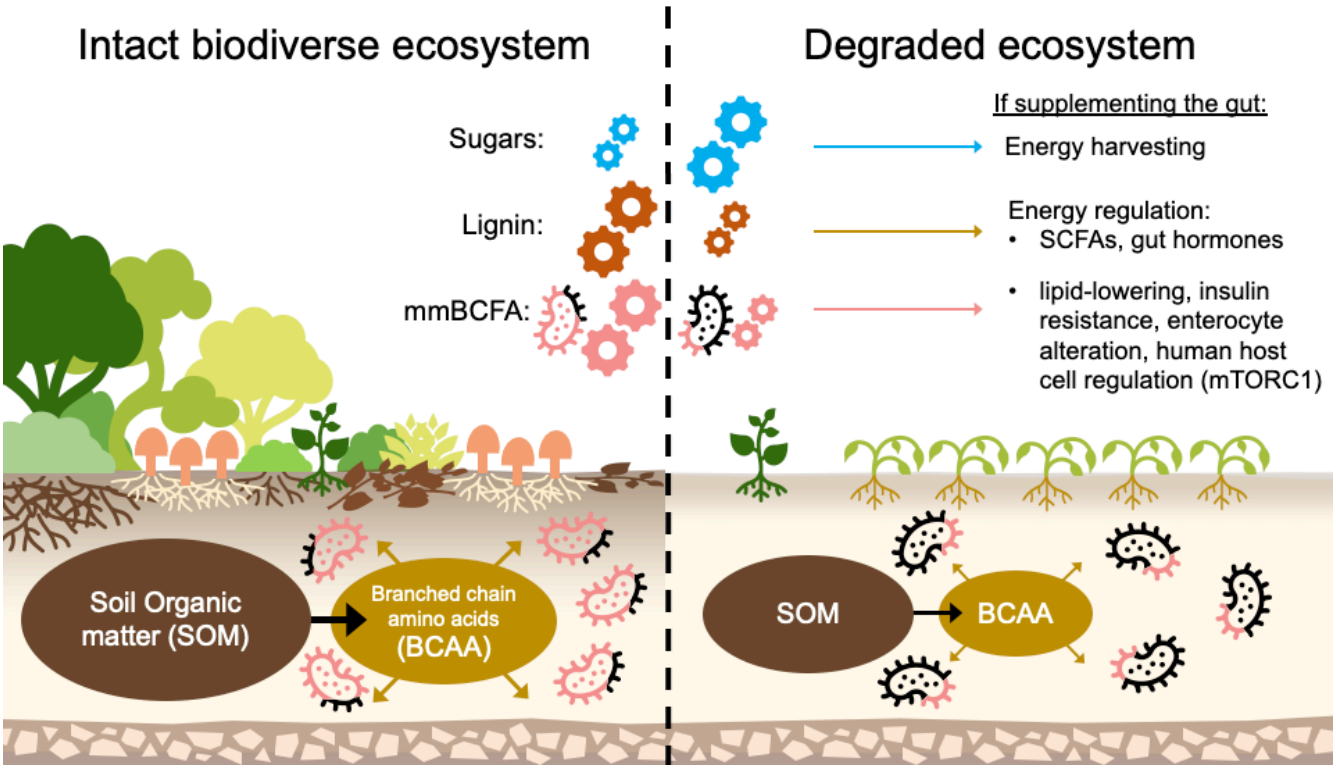

Figure 6. Hypothesis: Intact natural ecosystems typically have greater biodiversity, woody plant debris (including lignin), fungal recycling and soil organic matter (SOM) pools. SOM releases branched-chain amino acids (BCAA) which are used to make precious monomethyl branched-chain fatty acids (mmBCFA) 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 consume nutrient-depleted food (low mmBCFA and lignin content, high sugar content) and/or are increasingly exposed to soil microbiomes associated with degraded ecosystems, it may contribute to metabolic shifts in the gut consistent with type 2 diabetes.

601 ***Identified compounds linked to energy harvesting***

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

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

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

628

629 ***Identified compounds linked to energy management***

630 We found altered potential metabolism for compounds with plausible links to energy management
631 (e.g., energy storage via fatty acids, gut hormone secretion and appetite regulation) that decreased in
632 both degraded ecosystems and T2D. This finding supports the notion that nutrient-depleted foods
633 and/or excessive exposures to degraded ecosystem soils that are deficient in potential metabolism of
634 compounds involved in energy management may shape and supplement gut microbiomes that are also
635 deficient in this way, thereby contributing to further metabolic imbalances found in T2D. We discuss
636 above how gut microbiome mmBCFA content and biosynthesis capacity might modulate host cellular
637 metabolism via central mTORC1 regulation, alteration of enterocyte phospholipid composition and
638 function, and/or supply of BCFA for further host endogenous fatty acid synthesis and BCFA-mediated
639 metabolism.

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

649 coumaryl alcohol (both precursors to lignin) in the compound-wide results spanning all datasets.
650 Potential metabolism for pectin also decreased in T2D (significantly in the Swedish cohort, and
651 marginally in the Chinese cohort), but not in degraded ecosystems. We also searched for
652 oligosaccharides (e.g., fructan, inulin, galactan) but did not detect these compounds in our datasets.
653 Additional compounds that we identified remain to be explored (see Table S4).
654

655 ***Disentangling dietary and environmental influences on T2D gut microbiomes***

656 We expect that our results showing altered potential metabolism for compounds detected within gut
657 metagenomes may arise from a combination of environmental exposures that shape and supplement gut
658 microbiomes, dietary inputs that further support or deplete members of the gut microbiome, gut
659 environment and function, and neurological influences, among other factors (Cryan et al., 2019).
660 Where we found matching trends across all case studies (e.g., arabinose, fructose, melibiose, BCFA-
661 ACPs, lignin), this may be consistent with an alignment between environmental exposures and dietary
662 inputs. In the scenario where compounds consistently showed in both T2D cohorts, but not in the
663 restoration gradient, this suggests dietary effects alone. For example, increased potential metabolism
664 results for amylopectin suggests that excessive consumption of starch-rich foods may contribute to
665 T2D. For many compounds we found alignment in potential metabolism between the ecosystem
666 condition and T2D gut (Chinese cohort), but not the T2D gut (Swedish cohort) – this may point to
667 greater environmental exposures and detrimental health influence in some population groups. Yet
668 another contrast occurred in the case of ammonia, where potential metabolism trends were opposing in
669 the T2D cohorts, in the absence of any ecosystem condition effect, which suggests influence of dietary
670 exposures alone associating with T2D.
671

672 ***Implications for community connection to soils and ecosystems***

673 Our findings suggest that filling knowledge gaps in the pathogenesis of T2D may require more holistic
674 consideration of linkages, especially at the systems level of health risk and promotion (consistent with
675 Handte-Reinecker & Sardeshpande, 2025). For example, where landscape-scale ecosystem
676 management may drive changes in functional capacities of health-supporting microbiomes and
677 microbiome units of interest may span scales from collective activity of assemblages to constituent
678 compounds of microbial bodies. Our findings suggest that maintaining balanced functioning healthy
679 soil ecosystems in human habitats, especially those used for food production, is increasingly important
680 for supporting healthy human populations. Increasing urbanization, ecosystem degradation, the
681 industrialization of food production and its distribution, and reducing time spent in nature have
682 collectively reduced contact with soil microbiomes for a large proportion of people. When humans are
683 exposed to soil in modern environments, it is increasingly within urbanized or highly modified
684 landscapes (e.g., degraded ecosystems with low natural biodiversity, highly modified land and
685 disturbed soils, monocultural agricultural fields) where microbiomes and their functional capacities
686 have been greatly altered.

687 From a societal viewpoint, our findings suggest that addressing the global T2D pandemic may
688 benefit from restoring urban biodiversity, developing effective approaches for regenerative agriculture,
689 increasing soil biological health and diversity as key parts of a restorative continuum, and finding a
690 better balance between anthropogenic land use and nature. At the heart of this challenge lies the need
691 for a deeper understanding of soil health—recognizing soil as a complex living ecosystem in which the
692 microbiome plays a pivotal role. Understanding the critical links between soil health, food production,
693 and human health is fundamental for strengthening the diminished connections between people, land
694 and soil (Wall et al., 2015) and provides a foundation for developing strategies and actions that might
695 lead to improved gut health and resilience to T2D. This critical understanding of links between soil
696 ecosystem health and human health also draws on parallels of thinking about nature from an

697 Indigenous peoples' worldview. Indigenous knowledge offers guidance and support to theories that
698 place the health or condition of nature (land, biodiversity, water and air) as essential to maintaining
699 human health and wellbeing. Central concepts also recognize the importance of reconnecting with
700 nature by strengthening engagement with ecosystems through activities such as restoration and
701 regeneration. For example, in Aotearoa-New Zealand, Indigenous Māori stress connection and inter-
702 dependency with ecosystems through core principles such as ancestral links and association (Warbrick
703 et al., 2023). This includes concepts of whakapapa (genealogical descent of all living things) which
704 holistically reaffirms the importance of genetic and chemical assemblages in soils and landscapes to
705 support all life forms, including microbiomes (Harmsworth & Awatere, 2013). Concepts of
706 kaitiakitanga (guardianship of the environment) are also commonly used to reinforce actions and
707 strategies to guide environmental restoration and promote health. Indigenous perspectives offer
708 important guidance to all peoples for personally reconnecting and engaging with our ecosystems and
709 their stewardship in order to support healthy landscapes, soils, microbiomes and people.
710

711 ***Study limitations***

712 There is well documented regional geographic variation in soil and gut microbiomes (Andreu-Sánchez
713 et al., 2025; Delgado-Baquerizo et al., 2018; Kumar & Bhadury, 2023; Vasar et al., 2022) which may
714 affect the interpretation of our results. We have examined a limited number of case studies to
715 demonstrate a proof-of-concept, because relevant case study metagenome datasets (with publicly
716 available metadata that span gradients of ecosystem quality while limiting uncontrolled sources of
717 variation) are rare. Although, we note that land uses and management that degrade ecosystems may be
718 driving homogenization and hence greater predictability of soil microbiome structure and functions in
719 urban areas (Delgado-Baquerizo et al., 2021; Sun et al., 2025). Our method aims to bypass variation
720 that is often inherent with taxonomically focused studies, via targeting the hypothetical potential

721 metabolism of compounds, based on the idea that microbiomes will be universally shaped by the
722 resources (e.g., feedstocks) and the environmental conditions available to them. It remains to be tested
723 whether the patterns we observed hold in other geographic regions and human populations, and
724 whether they may be replicated under controlled experimental conditions. The SUPER-FOCUS and
725 ModelSEED resources we used have not been actively updated in recent years and may be missing the
726 latest functional annotations. There may be some inaccuracies in the mapping from SUPER-FOCUS
727 functions to ModelSEED pathways and reactions, however we use rule-based scripts that can be
728 refined, consistent with other software development. We recognize this CPP method represents a
729 simplification of complex metabolic processes within microbiomes (e.g., it does not discern anabolism,
730 catabolism, reactants or products). Moving beyond this proof-of-concept, future work should use
731 updated resources that enable comprehensive linkage from functional pathways to reaction and
732 compound databases. Noting the conceptual difference in our approach (CPP reflects functional
733 potential imprinted in DNA), we suggest future work should compare CPP measures to quantitative
734 measurement of compounds present (e.g., via metabolomics). Control samples were not included in the
735 case study datasets analysed, however all samples are considered 'high biomass' with low risk of
736 contamination, and we removed human genome sequences (observed at low levels) from gut
737 metagenome samples.
738

739 ***Conclusions***

740 We found overlapping trends in the potential metabolism of compounds with beneficial bioactivities
741 and involved in energy harvesting and management within separate model case study datasets of
742 degraded ecosystem soil from the United States and Australia, and T2D gut microbiomes from Swedish
743 and Chinese cohorts. Our results support existing studies implicating, at least in part, a role for
744 microbiomes in T2D. Additionally, our findings support the hypothesis that degraded soil microbiomes

745 may contribute to metabolic imbalances found in T2D via nutrient-depleted food (e.g., low mmBCFA
746 and lignin content, high sugar content) and/or excessive environmental exposures associated with
747 degraded ecosystems. Our novel method used to examine microbiome potential metabolism at the
748 resolution of individual compounds has derived new insights into potential links between soil,
749 ecosystem and T2D gut health that warrant further transdisciplinary investigation.
750
751

752 **Data availability**

753 Code used to support this study is available from: https://github.com/liddic/cpp3d_t2d . The datasets
754 used are available from NCBI Sequence Read Archive (accessions PRJEB1786 and PRJNA422434),
755 MG-RAST (project mgp16379), and soil metagenomes from the Australian Microbiome Initiative data
756 portal (https://data.bioplatforms.com/organization/australian-microbiome).

757

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