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Functional restructuring of the global soil microbiome under multiple stressors

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Abstract

Microbes, as the planet's most abundant and diverse organisms, drive soil functions globally and are vulnerable to environmental stressors triggered by global change. Yet, knowledge regarding the impacts of multiple environmental stressors on their functional profiles as well as the consequences for soil functionality largely remains unknown. Here, we analyze two global-scale datasets including information on soil metagenomics and multiple environmental stressors. We find that across terrestrial ecosystems worldwide, up to 60% of all functional genes significantly shift when soil microbes experience the high-level of concurrent stressors. In this regard, the relative abundances of genes involved in microbial growth are negatively linked to the increasing number of stressors. Conversely, those genes linked to stress resistance and energy production exhibit positive responses. Taken together, our findings highlight a significant restructuring of global soil functional microbiomes in response to multiple environmental stressors. Consequently, such restructuring drives community-level shifts in matter and energy reallocations, thereby impacting the maintenance of soil functionality under the projected global change.

Introduction

Soil microorganisms are among the most abundant and diverse organisms on Earth, and they play a critical role in regulating soil functioning and the delivery of key ecosystem services [1]. However, these organisms are highly vulnerable to global change [2,3]. Currently, soil microbiomes are subjected to an increasing number of environmental stressors triggered by climate change and anthropogenic activities, such as warming, drought, nitrogen deposition, increased salinity, pollution of heavy metals and organic chemical toxicants [4]. In this regard, soil microbial diversity is negatively influenced by an increase in such multiple stressors [5]. However, little information is known about how these multiple stressors reshape soil functional microbiome in natural ecosystems. The latest metagenomic study based on laboratory experiments indicated that multiple stressors affect soil microbes differently from individual factor alone [6]. This finding underscores the importance of studying concurrent environmental stressors. And further efforts could help evaluate whether microbial functional responses change proportionally with increasing multiple stressors and whether such stressors exert universal impacts across less controlled conditions and ecosystems. For example, metabolic strategies may be over-simplified in response to stress, which may result in a carbohydrate-driven metabolism that supports rapid microbial responses under low stress, as opposed to a more complex growth-driven metabolism under multiple stressors [7]. In this respect, understanding how soil microbial functional genes and pathways restructure under stress conditions is critical worldwide, as these changes could trigger cascading effects on terrestrial ecosystem services supporting human well-being, such as food production, climate regulation and soil fertility.

For now, two roadblocks hinder our understanding on effects of multiple stressors on the functional profile of soil microbiome, i.e. the comprehensive set of functional traits, metabolic pathways and associated ecological processed determined by soil microorganisms. First, no studies to date have simultaneously assessed the effects of multiple natural and anthropogenic environmental stressors on soil microbiomes across various regions and/or biomes. The meta-analyses can integrate the effect of single stressor at the global scale [8,9], but they are unable to capture the synergistic effects arising from the co-occurrence of multiple stressors. Similarly, simulated and/or controlled experiments [6,10] cannot account for the vast environmental heterogeneity present in natural ecosystems, thus limiting the ecological representativeness of conclusions. Second, most studies have focused on soil microbial taxonomic diversity while overlooking their functional traits, such as metabolic capability [5,8]. Microorganisms have developed sophisticated strategies to cope with environmental stresses, through regulating environmental information processing, cellular processes, genetic information processing and metabolism [10-12]. However, there are no empirical studies on specific positive or negative relationships for specific functions, such as carbohydrate metabolism and cell motility at a global scale, nor do we know how these relationships might vary across different biomes.

In this study, we employ a quantile approach to quantify the number of stressors that simultaneously exceed all possible quantile values (ranging from 5% to 95% stress levels based on the maximum observed levels for each stressor) [13]. This approach enables an in situ and ecologically representative assessment of the impacts of multiple stressors on soil functional microbiomes. We conduct a global survey including soil samples from 200 sites, across different biomes, spanning seven continents and encompassing a wide range of climates and vegetation types (e.g. forest, grassland and shrubland) (Fig. 1). It contains 23 individual attributes related to natural and/or anthropogenic stressors (details in Methods). These attributes are categorized into 10 groups, including aridity, maximum temperature, seasonality, extreme events, salinity, heavy

metals, soil pH, UV radiation, fire, and a synthetic human-influence index (Table S1). Then, we collect a global database deposited in NCBI that includes 1409 soil samples (Supplementary Fig. 1 and Supplementary data 1) [14] to further cross validate our results providing independent evidences. This database contains a smaller array of environmental stressors with 15 attributes affiliated within 8 groups. Microbial functional genes (Kyoto Encyclopedia of Genes and Genomes [KEGG] pathways and overall KEGG Orthologies [KOs]) [15] (see Supplementary Tables 2 and 3 for the detailed information on the categorization of KEGG pathways) are analyzed in response to the number of environmental stressors. We hypothesize that multiple stressors trigger a restructuring of the community-level functional profile of soil microbiome, particular in the metabolism categories. This suggests the alteration of stress-responsive genes and the acquisition of metabolic traits. This study will help predict the responses of soil microbiome to an increasing number of environmental stressors in an increasingly stressed world, and provide the novel evidence of their consequences on soil functionality and sustainability under projected global change.

Results and Discussion

Multiple stressors impact the community-level functional profiles of soil microbiome

Totally, there were 5299 KOs and 236 KEGG pathways observed in this study across 200 global ecosystems. The functional genes were compared between two groups, namely the one exposed to multiple stressors versus the other of unstressed control. Furthermore, we quantified the total number of genes, which exhibited significant changes in response to multiple stressors at low (25% quantile), medium (50% quantile) and high (75% quantile) levels (Table 1). Generally, the number of genes with decreased abundances was higher than the numbers with increased abundances at both medium and high stress levels (Table 1). Specifically, 20% of KOs and 32% of KEGGs significantly changed at the medium stress level, and 41% of KOs and 59% of KEGGs were at the high stress level (Table 1).

We used random forest modeling to identify which functional genes drove soil microbiome stressful responses and found that KOs and KEGG genes at level 2 were most responsive when exceeded low (25% quantile), medium (50% quantile) and high (75% quantile) levels of stress (Fig. 2). Meantime, the number of responsive functional genes was higher at medium or high levels than that at the low level (Fig. 2), indicating that the increase in stress level led to a broader functional gene response. For specific functional traits, when the stress level exceeded the medium level, 50% and 64% of responsive KOs and KEGG pathways were related to metabolism and these numbers became 38% and 50% at the high level (Fig. 2). The 1409-sample dataset gave the similar results: the top two KOs with the strongest response at the high stress level were both metabolism-related genes (Supplementary Fig. 2c). These findings suggest that metabolic reorganization is critical physiological processes for microbial community when exposed to multiple stressors simultaneously.

We further revealed the threshold, approximately the 30% quantile for responsive KEGG pathways (Fig. 3 and Supplementary Fig. 3). When stress levels exceeded this threshold value, KEGG pathways, such as carbohydrate metabolism, signaling molecules and interaction, transcription, transport and catabolism were positively correlated with the number of stressors ($P<0.05$), while these correlations became negative (Fig. 3) when below this threshold value. Meantime, those KEGG pathways, such as lipid metabolism, metabolism of other amino acids and cellular community-prokaryotes, exhibited contrary patterns, exhibiting positive correlations

below 30% quantile and negative ones when above (Fig. 3). The results of 1409-sample dataset were similar (Supplementary Fig. 3). This information suggests that soil microbiome can redirect their genomic investment along the stress-level gradient, via prioritizing metabolic pathways and environmental signal processing once over the medium stress level. It is supported by findings of the enrichment of microbial functional genes related to DNA repair, environmental sensing systems, antibiotic/secondary metabolite biosynthesis, and nutrient/osmoprotectant acquisition pathways under high levels of UV radiation, desiccation, and temperature extremes [16] and increased abundances of functional genes affiliated with cellular processes, genetic information processes and metabolism with rising temperatures [17]. However, as shown in Supplementary Fig. 4, the effects of individual stressors on the soil microbiome do not always follow a uniform direction of change. For instance, the genetic responses to multiple stressors (Multi_stressor_50 and Multi_stressor_75) align with those driven by aridity but differ from those associated with human influence, indicating that the effects of multiple stressors cannot be inferred simply by adding or exacerbating individual stressors.

Impaired metabolic pathways related to growth and reproduction are associated with multiple stressors

In view of the highest average relative abundance (Supplementary Table 2) and the most sensitiveness to stressing conditions (Fig. 2), we further focused on pathways within the metabolism category afterward. Meantime, given the distinct divergence in microbial responses across the 30% quantile (Fig. 3), subsequent analyses were focused on the 31–95% quantile interval, which corresponded to medium and high stress levels, the core stress gradients in this study.

It was found that soil microbiome underwent adjustments of a series of metabolic pathways. For example, declines in metabolic pathways related to microbial growth and reproduction processes were observed, as a response to an increase in the number of stressors. Specifically, KEGG pathways related to glycan biosynthesis and lipid metabolisms (at level 2) were negatively correlated with the number of stressors (Fig. 3 and Supplementary Fig. 5). At level 3, genes related to steroid hormone biosynthesis, lipopolysaccharide biosynthesis, as well as amino sugar and nucleotide sugar metabolism were all decreased (Fig. 4 and Supplementary Table 4). These genes are crucial for the formation of cell walls and membranes, which require high energy-containing carbon source and high number of ATP investment [18]. Furthermore, we observed a notable decline in pathways related to amino acid metabolism, including arginine and proline, beta-alanine, tyrosine, tryptophan, cysteine, methionine, and glutathione (Fig. 4). These pathways are tightly linked to nitrogen and sulfur metabolism, two essential elements in microbial cells and accounting for 12-15% and 0.5-1% of cell dry weight [19]. The reduction in the metabolism of these key amino acids can impair new protein synthesis and inhibit enzyme production and cellular growth [20,21]. Similarly, the depletion of methionine metabolism can lead to a halt in protein synthesis, because methionine is the initiating amino acid in mRNA translation process [22]. In addition, the reduction in these amino acids metabolism can affect the synthesis of other critical molecules. For instance, the suppression of β-alanine metabolism impedes the production of pantothenate and CoA, thereby disrupting acetyl-CoA-dependent lipid metabolism [23]. Cysteine deficiency compromises the synthesis of glutathione (GSH) and CoA, both of which are crucial for metabolism and stress signaling, contributing to metabolic dysregulation and impaired cellular growth [24]. The abundance of genes related to steroid hormone biosynthesis was negatively correlated with the number of stressors (Fig. 4). Steroids are essential components of eukaryotic cell membranes, which are

essential for membrane integrity and cellular stability [25]. In this regard, the reduction of steroid biosynthesis can lead to a decline in the biomass of eukaryotic organisms. These findings, consistent with results from pure-culture laboratory studies [26], provide community-level evidence that multiple stressors compromised the growth and reproduction of soil microbiomes.

Multiple metabolic pathways related to stress-defense are invested to help soil microbes survive under multiple stressors

Under stressful abiotic conditions, such as extremes of moisture, temperature, pH and salinity, microbial investment in activity maintenance is generally high [27]. Correspondingly, we found a positive correlation between the stressor number and fatty acid biosynthesis (Fig. 4) and a negative correlation with fatty acid degradation (Fig. 4). Meantime, severe stress boosted the pathways related to biosynthesis of unsaturated fatty acids (Fig. 4), especially arachidonic acid (Supplementary Fig. 3). Fatty acids are the key component of cell membranes, and the membrane fluidity attributes to the degree of fatty acid desaturation [28]. These phenomena suggest that microbes adopt the strategy of producing more fatty acids, particularly unsaturated fatty acids, to improve membrane fluidity and flexibility.

Similarly, amino acids metabolism is a well-known mechanism for biology to resist environmental stressors [29,30]. Therefore, we found that pathways involved in phenylalanine (Phe) synthesis (KEGG, at level 3) were most responsive in amino acids metabolism, and were positively correlated with the number of stressors (Fig. 4 and Supplementary Fig. 3). Beyond being a protein building block, Phe can serve as a precursor for vital plant compounds that support reproduction, growth, development, and stress defense [31]. Among thirteen tested amino acids, Phe is identified as the most effective one enhancing enzyme activities related to N cycling and promoting plant growth [32]. Phe metabolism is widely distributed and conserved genes of the phenylacetate pathway identified so far in 16% of the sequenced bacterial species [33]. This finding highlights the dual role of Phe as both a metabolic hub and a stress-response mediator under multiple stressors.

Furthermore, selenocompound metabolism was positively impacted by the number of stressors, while glutathione metabolism was negatively impacted (Fig. 4). Both compounds are important detoxification substances in response to stress [34,35]. Glutathione metabolism always increases under salt stress and oxidative damage in plants [36]. However, our findings are contrasting. This contradiction may be attributed to microbial energy conservation strategies under complex stress conditions. For example, selenoprotein metabolism connects the detoxification in microbes with the generation of proton motive force to drive cell growth [37], which serves as an energy-saving strategy.

Strengthened carbohydrate catabolism supplies adequate energy to soil microbiome under multiple stressors

As the number of stressors increases, the demand for energy rises. In this regard, we observed remarkable alterations in energy-generating metabolism. Specifically, genes associated with carbohydrate metabolism exhibited the strongest responsiveness, dominating both KEGG and KOs rankings (Fig. 2 and Supplementary Fig. 6). Among the metabolism category, 9 out of the top 10 pathways with the greatest responses belonged to carbohydrate metabolism (Fig. 4). Additionally, both datasets revealed that the pathways related to carbohydrate metabolism (for example 10 out of 15 in the 200-sample dataset, Fig. 4) were positively correlated with the number of stressors (Fig. 3 and Supplementary Fig. 5). Such prioritization suggests that carbohydrate metabolism acts as the important energy-hub for microbes responding to multiple stressors. Carbohydrates serve as

primary energy sources for microorganisms. The catabolic processes can ensure a continuous and reliable energy supply, vital for maintaining cellular functions and supporting repairing mechanism under stressful conditions [38]. For example, *Lactococcus lactis* subsp. *cremoris* increases its glycolytic rate under low pH [39]. Such metabolic prioritization is also observed in plant systems. Genes related to sugar transport, glucose catabolism and Krebs cycle are up-regulated in response to multifactorial stressors [40]. Our findings suggest that soil microbiome adopts the similar mechanism and such cross-kingdom convergence can be attributed to a fundamental bio-energy demand.

Our results suggested that when multiple stressors occurred, a universal multi-species coordination of soil microbiome can lead to community-level shifts in matter and energy reallocations. The energy expenditure may be ultimately irreconcilable among multi-species. Previous studies focused on the impact of a single stressor, such as salinity [41] and little evidence was found for such trade-offs among yield, stress, and resource acquisition at community level [42]. The positive relationship between stressing magnitude and microbial carbohydrate metabolism suggests universal bio-energetic constraints: environments with multiple stressors force microbiome to prioritize survival metabolism over growth processes, conserving energy and resources for resilience. Stressful conditions favor taxa with efficient metabolic streamlining and/or with alternative metabolic pathways [41]. Such kind of metabolic restructuring of microbiome can trigger cascading effects on soil ecological processes, e.g. accelerated carbohydrate decomposition and increased soil CO_2 emissions [43]. Therefore, increased concurrent stressors could lead to greater soil carbon losses than predicted by current global models [44]. This information highlights a knowledge gap in our understanding regarding microbial-mediated carbon cycling under multiple stressors triggered by global changes.

In conclusion, our metagenomic investigation of more than 1600 globally distributed soils revealed significant changes in the functional profile of microbiomes responding to multiple environmental stressors. Notably, up to 60% of genes and genome pathways tended to shift at the high-level of stressors, particularly from growth-oriented to maintenance-focused processes, which prioritized energy harvest and stress defense (Fig. 5). Such metabolic trade-off between growth and survival, aligning with plants, indicated the mechanistic convergence across phylogenetically divergent organisms. Yet, soil microbes tended to employ energy-efficient defense strategies, e.g. replacing pathways related to glutathione-dependent systems with phenylalanine and selenocompound metabolisms. In this regard, carbohydrate catabolism had to be strengthened to supply adequate energy. Taken together, our study revealed a global-scale generalized restructuring of soil functional microbiome in response to multiple environmental stressors, with the consequence for increased soil carbon losses and the potential challenge to soil sustainability under the projected global change.

Methods

Global field survey

In this study, we collected soil surface samples from 200 sites across various biomes worldwide (Fig. 1). These samples are a subset of samples of our previous surveys conducted between 2016 and 2019

[45-48]. These sampling sites included all dominant terrestrial ecosystems, i.e. temperate and tropical forests, boreal forests, temperate grasslands, subtropical deserts, subtropical woodlands and shrublands, ensuring a comprehensive representation of common environmental conditions found in terrestrial environments globally (the inset in Fig. 1). Site coordinates were recorded in situ using a portable GPS device, and vegetation types were identified on-site based on the dominant vegetation. At each site, a representative plot was established to reflect the local vegetation. To account for spatial heterogeneity in the plot, five soil cores were collected with a sampling depth < 10 cm (~0-10cm) after removing surface litter. The five cores were combined, placed in a zipped plastic bag and transported to the laboratory on ice. The soils were sieved (<2 mm) in the laboratory and divided into two subsamples: one was air-dried for soil physiochemical analyses, and the other was immediately frozen at −20 °C for molecular analyses. Further details of soil sampling are provided in Zhang et al. [2].

Samples were imported to Spain using letters of authority from the Ministerio de Agricultura, Pesca y Alimentación. After DNA extraction in Spain, DNA samples were imported to Australia for sequencing using permits from the Department Agriculture Fisheries and Forestry of Australia. Nagoya focal points from all involved countries were contacted to ensure we were following Nagoya regulations.

Metagenomic sequencing and bioinformatic analyses

Soil DNA was extracted from frozen soil samples using the Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Shotgun metagenomic sequencing was conducted at the Next Generation Sequencing (NGS) Facility (Western Sydney University, Australia) using the Illumina NovaSeq platform to generate approximately 10 Gb of 150 bp paired-end high-quality reads per sample. The clean reads were obtained after quality trimming using fastp (v0.20.1) to remove low-quality reads (with average quality scores < 20 and length < 50 bp).

Metagenomes were assembled using Megahit (v1.2.9). Only contigs ≥1000 bp were retained [49]. The open reading frames (ORFs) of assembled contigs were predicted using Prodigal (version 2.6.3). All ORFs were generated to a set of unique genes after clustering using CD-HIT [50]. The relative abundance of each predicted non-redundant gene was quantified as reads per million (RPM) using Salmon (v1.10.3) [51]. The functional annotations of the unique gene set in the metagenome-assembled genomes were searched against the eggNOG databases (v5.0) using eggnog-mapper (v2.1.12) [52]. Metagenomic coverage was estimated using Nonpareil v3.5.5 [53]. Although sequencing coverage was relatively low (ranging from 13.1% to 22.7%), this study focused on dominant functional genes of the soil microbiome, and the main conclusion were therefore not compromised. Future work with the deeper sequencing and higher coverage will further improve these analyses.

Groups of natural and anthropogenic environmental stressors

In this study, we worked with ten groups of environmental stressors (instead of multiple individual factors within each group) for two reasons. First, individual factors within each group (for example, within heavy metals) were highly correlated with each other, suffering from multicollinearity. Second, different groups of environmental stressors comprised a different number of individual factors. These ten groups were selected on the basis of two criteria: their well-known importance and data availability in the used databases. The ten groups did not suffer from statistical multicollinearity presenting different types of stressors, and they reflected largely independent

statistical entities.

We considered ten common environmental factors that can result in stress when passing high levels. They were aridity (inverse of aridity index), maximum temperature, seasonality (precipitation and temperature seasonality and diurnal temperature range), extreme events (extreme dry events, extreme wet events, heatwave days), fire, salinity, soil pH (distance from neutral pH), levels of heavy metals (soil As, Cd, Cr, Cu, Ni, Pb and Zn), human influence (for example, human-influence index and fertilization) and UV radiation.

Temperature and seasonality variables were obtained from WorldClim (v.2) (http://www.worldclim.com/version2). Aridity Index was calculated as the ratio of precipitation to potential evapotranspiration [2]. Electrical conductivity was measured in a 1: 5 (w/v) soil-to-water extract using a conductivity meter and used as a proxy for soil salinity [54]. Soil pH was measured in a 1: 2.5 (w/v) soil-to-water extract with a pH meter. The concentration of As, Cd, Cr, Cu, Ni, Pb and Zn were quantified as a measured of heavy metal concentration using inductively coupled plasma optical-emission spectrometry with (Thermo ICP 6500 Duo equipment) before microwave digestion [4]. The human influence index included data on human influence (a global dataset of 1 km grid cells derived from nine global data layers covering human population pressure [55] as well as information on human-induced nitrogen (N) deposition from fertilizer application and manure [56,57].

Calculation of the number of environmental stressors

We initially standardized the values associated with each environmental stressor (e.g. aridity index, extreme events or seasonality) to a range of 0 to 1 across all sites. For instance, the site(s) with the highest level of aridity was assigned a value of 1, whereas the site(s) with the lowest level of aridity received a value of 0. This standardization eliminates the effects of differences in measurement scales between variables, and creates a metric that is intuitively interpretable [13]. We then averaged the values of the environmental stressors within each stressor group (for instance, mean diurnal temperature range, temperature and precipitation seasonality values were averaged to determine seasonality). We then investigated how many target stressors exceeded one certain quantile. For instance, when setting the quantile at 50%, we counted the number of stressors with standardized values above 0.5 for each site. Each site thus had a corresponding number of environmental stressors at the quantile 50%. In this study, we opted to include all possible levels because the selection of quantiles was arbitrary, i.e. the different quantiles here did not have specific ecological meaning. The 5% and 95% quantiles were used to define the minimum and the maximum levels to avoid outliers. More details can be seen in Rillig et al. [4].

Cross-validation using the 1409-sample dataset

To evaluate the reliability and validity of the findings derived from the global field survey, we employed a dataset comprising 1409 samples (Supplementary Fig. 1) distributed globally, which was collected from NCBI [14]. This dataset encompassed a diverse range of ecosystem types on the global scale, including alpine soil, bare land, desert, forest, grassland, shrubland, tundra and volcanic soil.

This dataset contained eight groups of environmental stressors: aridity, maximum temperature, seasonality, extreme events, fire, soil pH, human influence and UV radiation, but without heavy metals and salinity. The definition and standardization of their occurrence, as well as the calculation of the number of stressors were consistent with those applied in the global field survey. Metagenomic assembly and the functional annotations were performed using the same

protocols as in the initial study.

Statistical analyses

The read counts of functional genes, including KOs and KEGG pathways at level 3 (Supplementary Table 3) were analyzed to identify genes with significant changes in response to multiple stressors. At the low (25%), medium (50%) and high (75%) stress levels, the 200 sites were categorized into two groups based on the number of stressors (n): one group with multiple stressors (n > 0) and the other without multiple stressors (n = 0). The non-rarefied feature tables were input into the DESeq2 R package using default settings [58]. Genes with zero read counts across all samples were removed. Differential expression analysis was performed using methods based on the negative binomial distribution. Read counts were modeled as negative binomial distributions with estimated mean values and gene-specific dispersion parameters, with each gene being fitted as a generalized linear model. Finally, Wald statistics and the Benjamini-Hochberg and false discovery rate (FDR) correction were applied to assess significance and multiple comparisons.

Random forest regression analysis [59] was conducted to model the response of functional genes (KO gene structures and KEGG pathways) to the number of stressors exceeding 25%, 50%, and 75% quantiles. Random forest analyses create a collection of classification trees with binary splits, fitting each tree using randomly selected cases (one-third of the data) that are withheld during its construction (out-of-bag cases). The importance of each predictor variable is quantified by measuring the reduction in prediction accuracy (i.e., the increase in mean square error between observations and out-of-bag predictions) when the data for that predictor is randomly permuted [13].

Negative or positive correlations between functional genes within metabolism at levels 2 and 3 and the number of environmental stressors (using quantiles of 5-95%, iteratively increasing by 1%) were identified using non-parametric Spearman correlations. Spearman rank correlations measure the direction and strength of the association between two ranked variables and do not require normality of data or homogeneity of variances. A two-tailed test p-value < 0.05 was considered statistically significant.

We selected all dominant functional genes (at level 3) associated with metabolism that had a relative abundance greater than 0.1%, which were listed in Supplementary Table 4. The correlation coefficients from the Spearman correlations analysis of dominant functional genes and the number of environmental stressors (using quantiles of 31-95%; Supplementary Table 4) were summed to obtain the total r value. The top 30 functional genes with the highest absolute r values were then selected to draw stick plot.

Random forest regression, heatmaps and stick plots were drawn using the rfPermute, pheatmap and ggplot2 packages in R version 4.3.0.

Data Availability

All environmental variables for both the 200-sample and 1409-sample datasets used in this study have been deposited in Figshare [https://doi.org/10.6084/m9.figshare.31817422].

The metagenomic sequences for the 200-sample dataset have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA1162941 [https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA1162941]. The corresponding SRA run numbers for the 200-sample dataset are provided along with the environmental variables in Figshare [https://doi.org/10.6084/m9.figshare.31817422].

The metagenomic sequences for the 1409-sample dataset are compiled from NCBI SRA, and the corresponding SRA run numbers are provided in Supplementary Data 1.

Code Availability

The metagenomic bioinformatics analysis scripts and all R codes in this study are available on Figshare at https://doi.org/10.6084/m9.figshare.31817422.

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Author Contributions Statement

Conceptualization: M.D.-B., B.K.S., F.T.M., Y.F. and B.M.; Investigation: R.C., S.L., Y.F., F.T.M., T.S.-S., N.G., Y.L.B.-P., V.O., B.G., E.G., M.G.-G., E.V., S.A. (Sergio Asensio), J.M.-V., B.J.M., S.A. (Sebastian Abades), F.A., M.B. (Matt Barrett), M.B. (Miguel Berdugo), J.L.B.P., N.B., B.B., M.B. (Matthew Bowker), H.C. (Helena Castro), H.C. (Haiyan Chu), N.A.C., Z.D., B.D., J.D., C.I.E., A.F. (Alex Fajardo), K.F., A.F. (Ana Foronda), L.F., K.G., T.G., E.G.M., S.C.H., L.K., M.K., L.L., P.C.L.R., P.L., A.L., M.A.L., P.M., G.M.-K., T.M., A.M., E.M., D.M., J.P.M., G.M., S.M., M.M.-R., G.N., S.N., A.N., C.P., Y.P., P.J.R., A.R. (Ana Rey), A.d.l.R., A.R. (Alexandra Rodríguez), B.R.L., R.R., J.R., A.S., J.S., H.L.T., S.T., T.U.N., M.U., O.V., L.W., M.A.W., C.X., J.X., E.Z., B.M., B.K.S., and M.D.-B.; Methodology and visualization: R.C., S.L., Y.F., C.X., T.S.-S., B.M., and M.D.-B.; Writing-reviewing & editing: R.C., S.L., Y.F., F.T.M., C.X., T.S.-S., B.M., B.K.S., and M.D.-B. with contributions of all authors.

Competing Interests Statement

The authors declare no competing interests.

Tables

Table 1. The number of genes exhibited significant changes in response to multiple stressors at low, medium and high stress level. The number of all genes was identified at level 3 for both KOs and KEGG. The 200 sites were classified into two groups based on the number of stressors (n): the group with multiple stressors (n>0) and the group without multiple stressors (n=0). Stressor levels were assessed at 25%, 50% and 75% quantiles of maximum observed values. The number of genes with significant changes between the two groups was calculated using DESeq2 based on the read counts of genes. Values in parentheses represent the proportion of target genes relative to the total number of genes. At the low stress level (25% quantile), the number of stressors were over 0 for all the sites.

	KOs			KEGG		
Stress level (quantile)	Low (25%)	Medium (50%)	High (75%)	Low (25%)	Medium (50%)	High (75%)
Number of all genes		5299			236	
Number of genes with significant changes	/	1065 (20%)	2153 (41%)	/	77 (32%)	138 (59%)
Number of genes with increased abundance	/	508 (9.6%)	905 (17%)	/	36 (15%)	65 (28%)
Number of genes with decreased abundance	/	557 (10.5%)	1248 (24%)	/	41 (17%)	73 (31%)

Figure Legends

Figure 1| Locations of samples in this study. Points denote 200 samples in a global survey with 10 groups of environmental stressors (aridity, maximum temperature, seasonality, extreme events, fire, soil pH, human influence, UV radiation, heavy metals and salinity). The inset shows the distribution of 200 samples among climate (sub) zones based on Whittaker biomes.

Figure 2| Main functional genes associated with the number of environmental stressors simultaneously exceeding 25%, 50%, and 75% quantiles. The x-axis (%IncMSE) indicates the increase of the mean squared error when the given variable is randomly permuted. Orange bars indicate significant variables at the level of 0.05 (two-sided statistical test). The inset table shows the number of responsive genes under different stress scenarios, with each column corresponding to respective quantile in the above figure. Colors in the left table represent the category information of level 1 and level 2, with detailed category information provided in Supplementary Table 2. KEGG, Kyoto Encyclopedia of Genes and Genomes pathways. KOs, and overall KEGG Orthologies. Env info pro, Environmental information processing. Gen info pro, Genetic information processing. Cellular pro, Cellular processing.

Figure 3| Shifts of functional profile with the increase in the number of environmental stressors. The number of environmental stressors was calculated over quantile levels ranging from 5% to 95% stress levels based on the maximum observed levels for each stressor. Correlations with a false discovery rate adjusted $P > 0.05$ are excluded (plotted in white, two-sided statistical test). The color intensity indicates the strength of the correlation, with blue representing a negative correlation and red for a positive correlation.

Figure 4| KEGG pathways associated with metabolism showing significant responses to multiple environmental stressors. Data are the sum of the correlation coefficients between gene abundance (KEGG associated with metabolism) and the number of environmental stressors, with quantile levels ranging from 31% to 95%. The figure displays the 15 KEGG pathways with the highest positive correlations and the 15 with the highest negative correlations. KEGG, Kyoto Encyclopedia of Genes and Genomes pathways. The detailed category information of KEGG was listed in Supplementary Table 2 and Supplementary Table 3. Different colors denote the category information of KEGG pathways at level 2. The number near the circle was the means of relative abundance of each KEGG pathway. Detailed data can be found in Supplementary Table 4.

Figure 5| A conceptual diagram summarizing shifts in functional profile of the soil microbiome in terrestrial ecosystems under multiple environmental stressors. At the medium stress level, 32% of KEGG significantly changed, among them 15% with increased abundance and 17% with decreased abundance. At the high stress level, 59% of KEGG significantly changed, among them 28% with increased abundance and 31% with decreased abundance. With an increasing number of stressors simultaneously exceeding medium-high levels of stress, microbial metabolism shifted from growth processes (e.g. syntheses related to glycan, lipid, amino acids, amino sugar and sulfur) to energy production (e.g. carbohydrate degradation) and stress-defense mechanism (e.g. unsaturated fatty acids, phenylalanine and selenocompound). Blue indicates a decrease and red indicates an increase. Color intensity represents the strength of the response. Arg, arginine; Pro, proline; Cys, cysteine; Met, methionine; Try, tryptophan.

Editorial summary:

Soil microbes drive ecosystem functions but are vulnerable to environmental stressors triggered by global change. This study reveals that multiple environmental stressors drive community-level restructuring of soil functional microbiomes globally.

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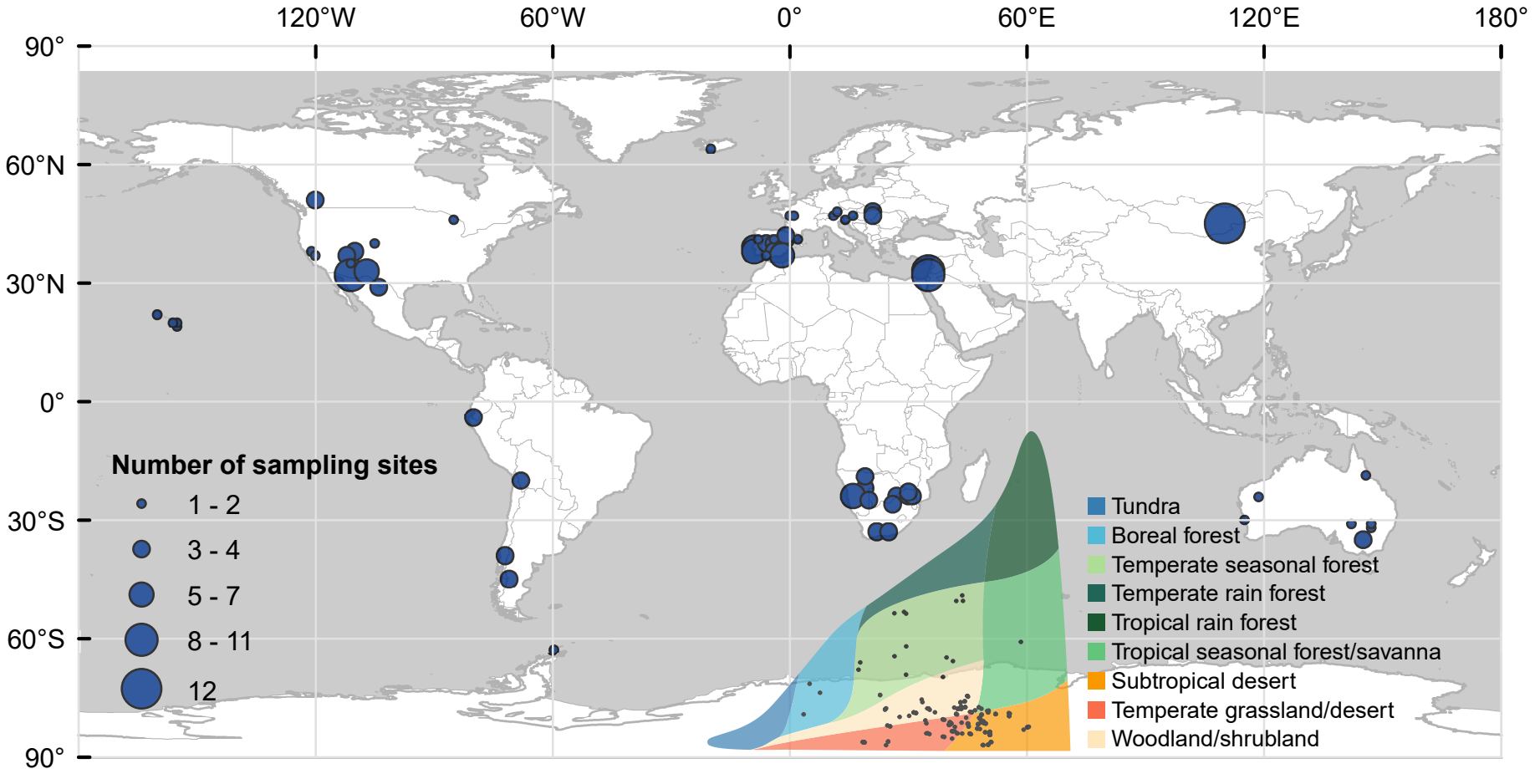
120°W
60°W
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90°
60°N
30°N
0°
30°S
60°S
90°
Number of sampling sites
1 - 2
3 - 4
5 - 7
8 - 11
12
Tundra
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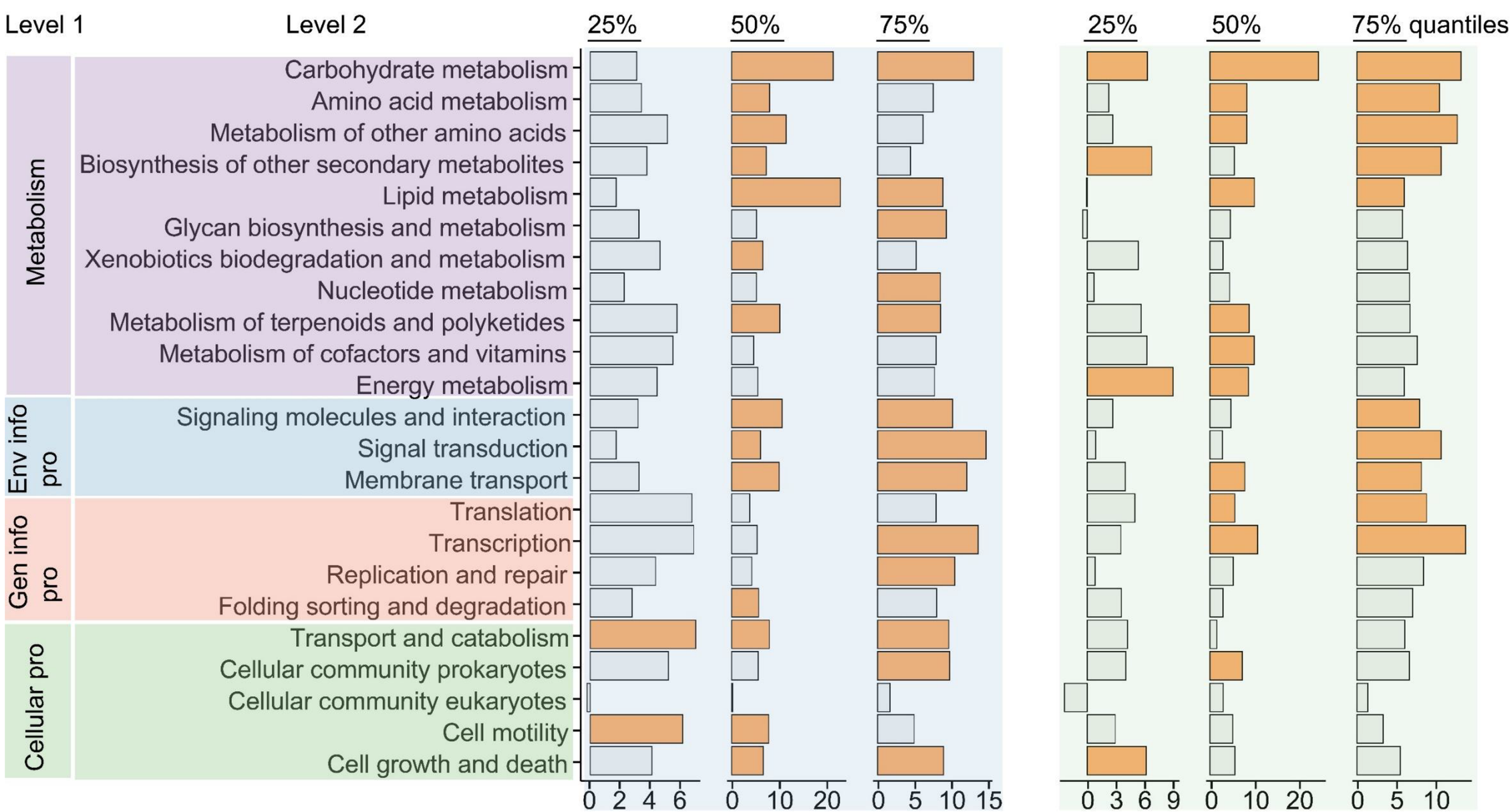
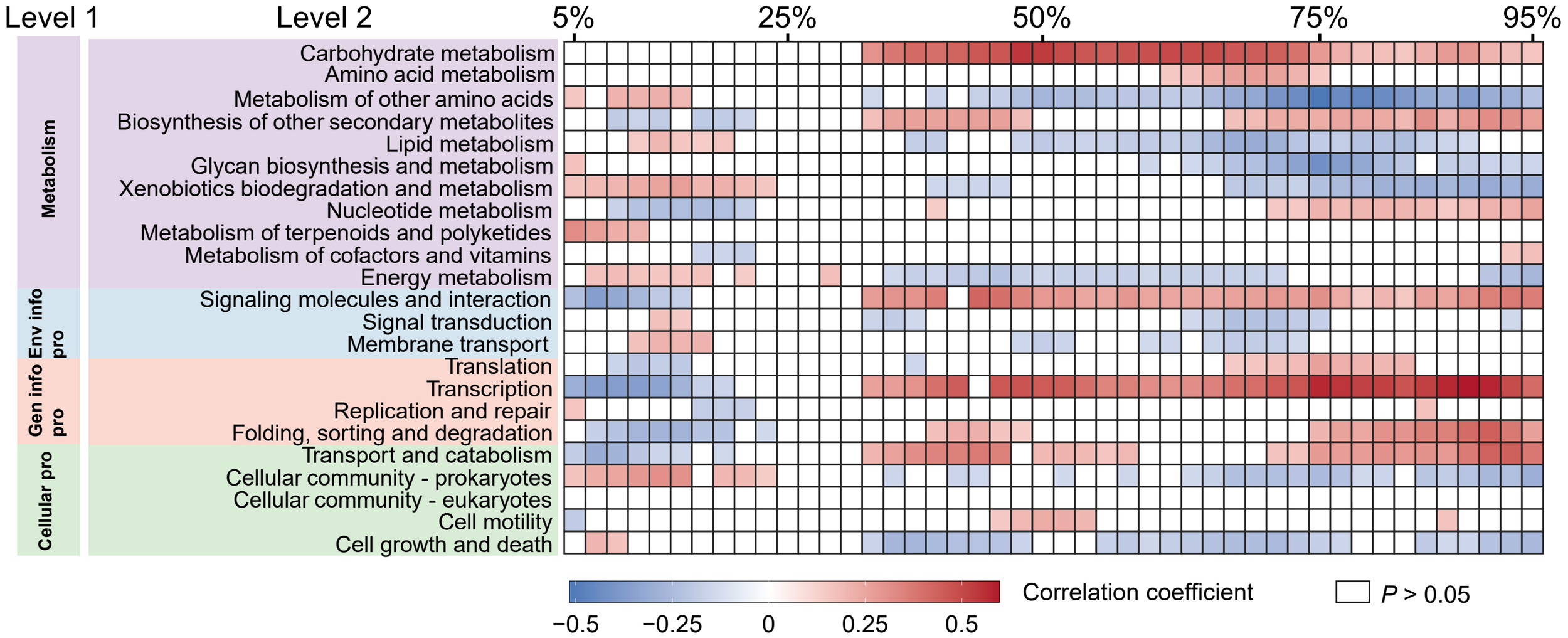
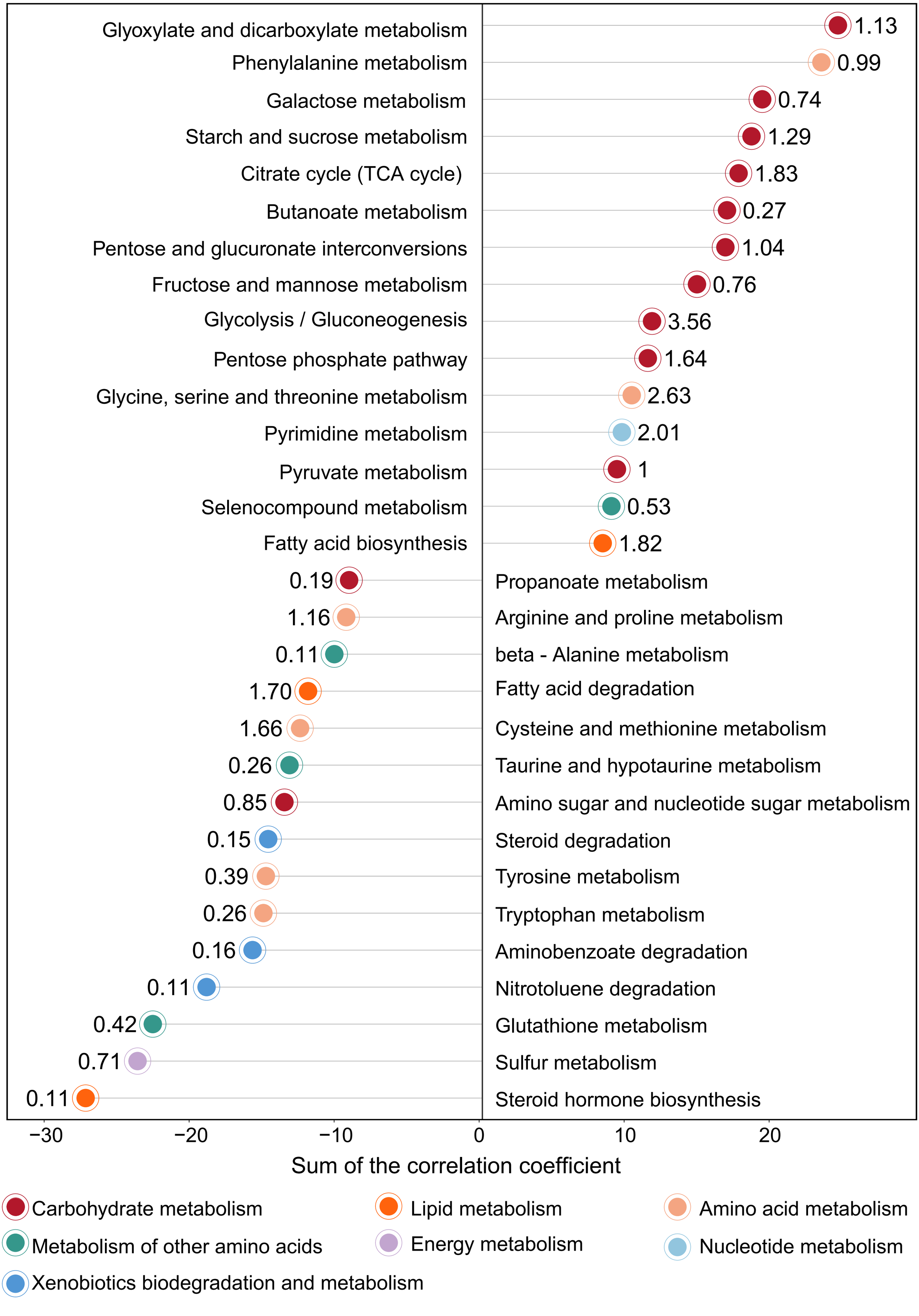
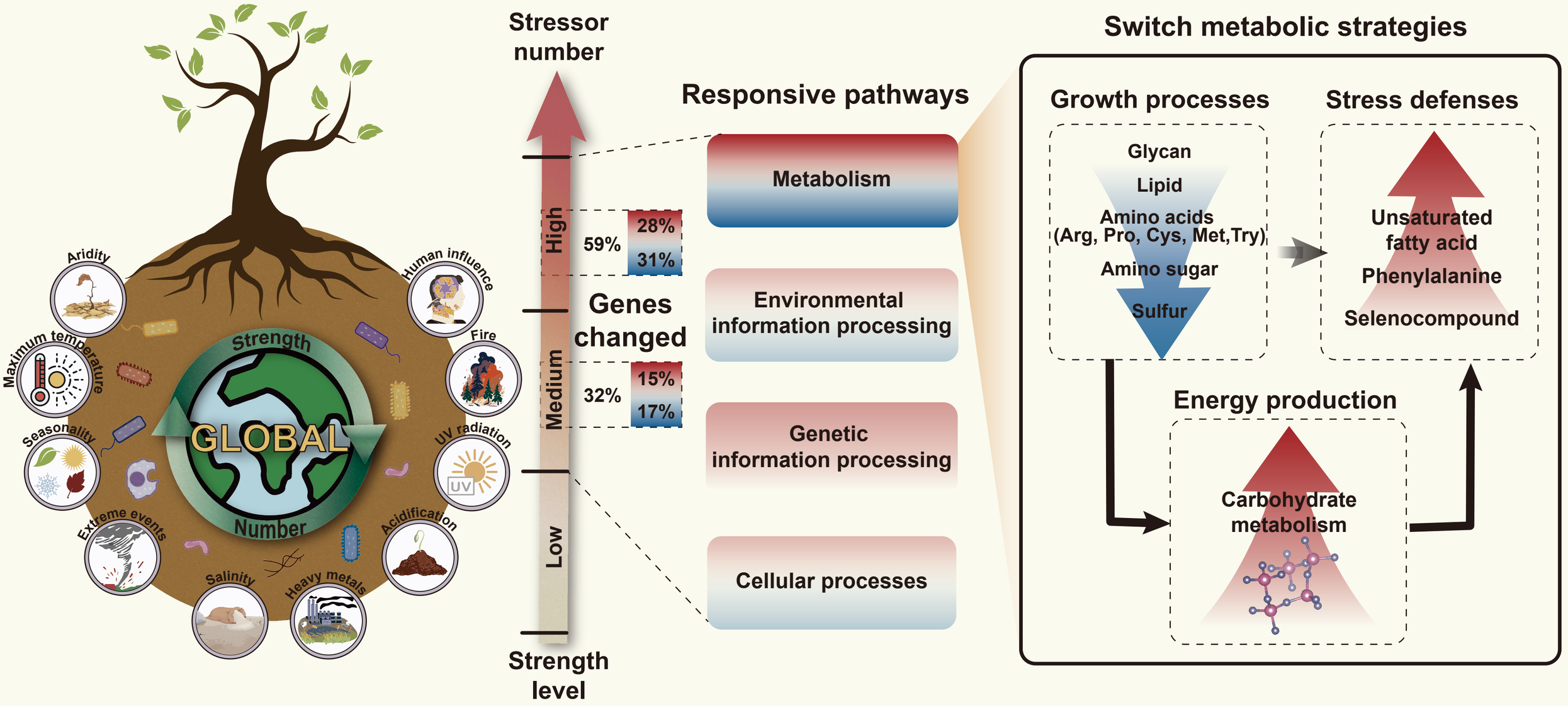
Temperate seasonal forest
Temperate rain forest
Tropical rain forest
Tropical seasonal forest/savanna
Subtropical desert
Temperate grassland/desert
Woodland/shrubland



Number of responsive genes	KOs			KEGG		
Total	2	14	13	4	11	10
Metabolism	0	7	5	3	7	5
Ratio	0	50%	38%	75%	64%	50%


Level 1
Level 2
5%
25%
50%
75%
95%
Metabolism
Env info pro
Gen info pro
Cellular pro
Carbohydrate metabolism
Amino acid metabolism
Metabolism of other amino acids
Biosynthesis of other secondary metabolites
Lipid metabolism
Glycan biosynthesis and metabolism
Xenobiotics biodegradation and metabolism
Nucleotide metabolism
Metabolism of terpenoids and polyketides
Metabolism of cofactors and vitamins
Energy metabolism
Signaling molecules and interaction
Signal transduction
Membrane transport
Translation
Transcription
Replication and repair
Folding, sorting and degradation
Transport and catabolism
Cellular community - prokaryotes
Cellular community - eukaryotes
Cell motility
Cell growth and death
−0.5
−0.25
0
0.25
0.5
Correlation coefficient
P > 0.05


Glyoxylate and dicarboxylate metabolism
1.13
Phenylalanine metabolism
0.99
Galactose metabolism
0.74
Starch and sucrose metabolism
1.29
Citrate cycle (TCA cycle)
1.83
Butanoate metabolism
0.27
Pentose and glucuronate interconversions
1.04
Fructose and mannose metabolism
0.76
Glycolysis / Gluconeogenesis
3.56
Pentose phosphate pathway
1.64
Glycine, serine and threonine metabolism
2.63
Pyrimidine metabolism
2.01
Pyruvate metabolism
1
Selenocompound metabolism
0.53
Fatty acid biosynthesis
1.82
0.19
Propanoate metabolism
1.16
Arginine and proline metabolism
0.11
beta - Alanine metabolism
1.70
Fatty acid degradation
1.66
Cysteine and methionine metabolism
0.26
Taurine and hypotaurine metabolism
0.85
Amino sugar and nucleotide sugar metabolism
0.15
Steroid degradation
0.39
Tyrosine metabolism
0.26
Tryptophan metabolism
0.16
Aminobenzoate degradation
0.11
Nitrotoluene degradation
0.42
Glutathione metabolism
0.71
Sulfur metabolism
0.11
Steroid hormone biosynthesis
−30
−20
−10
0
10
20
Sum of the correlation coefficient
Carbohydrate metabolism
Lipid metabolism
Amino acid metabolism
Metabolism of other amino acids
Energy metabolism
Nucleotide metabolism
Xenobiotics biodegradation and metabolism


Aridity
Human influence
Maximum temperature
Fire
Strength
GLOBAL
Number
Seasonality
UV radiation
UV
Extreme events
Acidification
Salinity
Heavy metals
Stressor number
High
Medium
Low
Strength level
59%
28%
31%
Genes changed
32%
15%
17%
Responsive pathways
Metabolism
Environmental information processing
Genetic information processing
Cellular processes
Switch metabolic strategies
Growth processes
Glycan
Lipid
Amino acids (Arg, Pro, Cys, Met,Try)
Amino sugar
Sulfur
Stress defenses
Unsaturated fatty acid
Phenylalanine
Selenocompound
Energy production
Carbohydrate metabolism