

Sustainable irrigation reduces arsenic bioavailability in fluvio-alluvial soils promoting microbial responses, high rice productivity and economic profit

Arnab Majumdar^{1,2*}, Munish Kumar Upadhyay³, Biswajit Giri², Debojyoti Moulick⁴, Sukamal Sarkar⁵, Barun Kumar Thakur⁶, Poonam Yadav⁷, Kashinath Sahu⁸, Ashish Kumar Srivastava⁹, Martin Buck¹⁰, Mark Tibbett¹¹, Manoj Kumar Jaiswal², Tarit Roychowdhury¹

¹School of Environmental Studies, Jadavpur University, Kolkata-700032, India

²Department of Earth Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal 741246, India

³Centre for Environmental Science & Engineering, Department of Civil Engineering, Indian Institute of Technology Kanpur-208016, India

⁴Department of Environmental Science, University of Kalyani, Nadia, West Bengal, 741235, India

⁵School of Agriculture and Rural Development, Ramakrishna Mission Vivekananda Educational and Research Institute, Ramakrishna Mission Ashrama, Narendrapur, Kolkata – 700103, India

⁶Department of Economics, FLAME University, Pune, Maharashtra-412115, India

⁷Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, Uttar Pradesh-221005, India

⁸Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal 741246, India

⁹Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra- 400085, India

¹⁰Department of Life Science, Faculty of Natural Sciences, Imperial College, London, SW7 2AZ, UK

¹¹Department of Sustainable Land Management and Soil Research Centre, School of Agriculture Policy and Development, University of Reading, Reading, RG6 6AR, UK

*Corresponding author- Dr. Arnab Majumdar (arnabmajumdar891@gmail.com, geomicrobiology.arnab@gmail.com)

This manuscript is a non-peer-reviewed preprint submitted to EarthArXiv. At present, this manuscript is submitted to ACS Environmental Science & Technology.

Author details-

Arnab Majumdar*

School of Environmental Studies, Jadavpur University, Kolkata-700032, India

Department of Earth Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal 741246, India

Email- arnabmajumdar891@gmail.com, geomicrobiology.arnab@gmail.com

Twitter handle- <https://twitter.com/ArnabMa41557200>

Munish Kumar Upadhyay

Centre for Environmental Science & Engineering, Department of Civil Engineering, Indian Institute of Technology Kanpur-208016, India

Email- munish007up@gmail.com

Biswajit Giri

Department of Earth Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal 741246, India

Email- giri.biswajit605@gmail.com

Debojyoti Moulick

Department of Environmental Science, University of Kalyani, Nadia, West Bengal, 741235, India

Email- drubha31@gmail.com

Sukamal Sarkar

School of Agriculture and Rural Development, Ramakrishna Mission Vivekananda Educational and Research Institute, Ramakrishna Mission Ashrama, Narendrapur, Kolkata – 700103, India

Email- sukamalsarkarc@yahoo.com

Barun Kumar Thakur

Department of Economics, FLAME University, Pune, Maharashtra-412115, India

Email- barun.thakur@flame.edu.in

Poonam Yadav

Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, Uttar Pradesh-221005, India

Email- poonamyadav.b.h.u@gmail.com

Kashinath Sahu

Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal 741246, India

Email- getkashi2011@gmail.com

Ashish Kumar Srivastava

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra- 400085, India

Email- ashishbrc@gmail.com

Martin Buck

Department of Life Science, Faculty of Natural Sciences, Imperial College, London, SW7 2AZ, UK

Email- m.buck@imperial.ac.uk

Mark Tibbett

Department of Sustainable Land Management and Soil Research Centre, School of Agriculture Policy and Development, University of Reading, Reading, RG6 6AR, UK

Email- m.tibbett@reading.ac.uk

Manoj Kumar Jaiswal

Department of Earth Sciences, Indian Institute of Science Education and Research (IISER)

Kolkata, Mohanpur, West Bengal 741246, India

Email- manoj@iiserkol.ac.in

Tarit Roychowdhury

School of Environmental Studies, Jadavpur University, Kolkata-700032, India

Email- rctarit@yahoo.com

Abstract

To minimize arsenic (${}^{74.92}\text{As}^{33}$) loading into rice plants, based on our two-year field trial we propose adopting alternating wetting and drying (AWD) irrigation as a sustainable water management strategy allowing greater silicon (${}^{28.08}\text{Si}^{14}$) availability. Our field-based project is the first report on AWD's impact on As-Si distribution in fluvio-alluvial soils of the entire Ganga valley (24 study sites, six divisions), seasonal variance (pre-monsoon and monsoon), rice plant anatomy and productivity, soil microbial diversity, microbial gene ontology profiling and associated metabolic pathways. Under AWD to flooded and pre-monsoon to monsoon cultivations, respectively, As-bioavailability was reduced by 8.71 ± 0.014 – $9.25\pm 0.019\%$ and 25.68 ± 0.088 – $26.13\pm 0.0921\%$. In the pre-monsoon and monsoon seasons, the rice grain yield under AWD improved by $8.41\pm 0.075\%$ and $9.96\pm 0.069\%$, proving the economic profitability. Compared to waterlogging, AWD evidences as an optimal soil condition for supporting soil microbial communities in rice fields, allowing diverse metabolic activities, including As-resistance, and active expression of As-responsive genes and gene products. Finally, we propose that by using AWD, the use of groundwater can be reduced, lowering the cost of pumping and field management and generating an economic profit for farmers. These combined assessments prove the acceptability of AWD for the establishment of multiple sustainable development goals (SDGs).

Keywords- Alternate wetting-drying; sustainable agriculture; rice productivity; economic profit; high throughput sequencing; gene ontology; microbial metabolism

Synopsis statement- Sustainable water management in rice cultivation reduces arsenic contamination and increases productivity while promoting soil microbial metabolic activities, gene expressions and economic profit for the farmers.

■ Introduction

In the soil-water-rice agro-ecosystem, both geogenic as well as anthropogenic factors contribute to arsenic (As) build-up in paddy fields¹⁻³. However, the As-hotspot areas spread across the globe, where the As content was reportedly exceeding permissible limits^{4,5}. Multifactorial influence (e.g., rice variety, soil elemental biogeochemistry, agro-environmental parameters, agronomic practices etc.) ultimately determines the susceptibility of rice varieties to As stress^{6,7}. The bioavailability of As is usually governed by pH, the proportion of cationic and anionic species, its association with soil minerals as well as the actions of the soil microbiome⁸. Considering rice production on a global scale, observations have projected

demand of 550.0 Mt rice production to cope with the issue to feed increasing global rice consumers and maintain the pace of economic growth too⁹. For optimal growth and yield, rice plant physiology and structural integrity must tolerate adverse environmental effects, made possible by high silicon (Si) accumulation in rice plants¹⁰. As and Si are structurally similar and inversely correlated¹¹. Under frequent alterations of soil pH and redox state, the release of labile soil Si can occur¹², ready for rice plant uptake. Flooded conditions in rice cultivation results in significant water loss through seepage, surface runoff, evaporation, and deep percolation¹³. However, alternate wetting and drying (AWD) was found to be sufficient in minimizing As load in grain by reducing the bioavailability of As to rice plants leading to reductions in As translocation into the above-ground part^{14,15}. This AWD practice can greatly influence soluble Si percentage and also influence soil microbial communities to thrive^{11,16}. The Gangetic Plain is one of the enriched fluvial-alluvial valleys in the world producing the bulk of India's rice^{17,18}. Loading of As in rice needs to be managed while increasing productivity and future food storage. A fluvio-alluvial soil can harbour diverse microbial communities that are naturally resistant towards As^{19,20}, and under differential soil status, microbial communities can express their complex network of metabolic activities relevant to As stress and agro-environmental practices^{21,22}.

In the fluvial-alluvial soil, As and Si dynamics are yet to be understood together in the context of rice productivity, socio-economic profit to the farmers, soil health quality via microbial active metabolisms and their diversity. This study is the first report from the Indian subcontinent involving the whole Ganga Valley to establish proposed practices in rice cultivation. Objectives of this study were to define- (i) As and Si status and distribution pattern in fluvio-alluvial soils of different geographic and topographic locations, (ii) how AWD practice can make a difference in As bioavailability in the soil to rice plants when Si is counteracting in the field, (iii) generated profit in AWD practice from the difference of rice productivity and minimized water usage, (iv) soil microbial community alterations under AWD and their expression of active metabolic pathways, (v) triggered As-resistance ability in soil microbes under AWD and helping in the reduction of As bioavailability in the soil. The project specifically was designed to unite many Sustainable Development Goals SDGs specifically SDG 2, SDG 3, SDG 6, SDG 9, SDG 12 and SDG 15²³. Outcomes allow the promotion of SDGs based on holistic sets of field study data applicable to global SDGs perspectives as well, as discussed in this report.

■ **Materials and method**

Sampling site selection and experimental design. Soils at the bank of Ganga are mostly fluvial-alluvial type²⁴ and allow easy translocation of As from soil to rice plants while cultivated anaerobically²⁵. The whole Gangetic plain was divided into six sections including four states- upper Ganga (Uttarakhand), upper-middle Ganga (Uttar Pradesh), middle Ganga (Uttar Pradesh), middle Ganga (Bihar), middle-lower Ganga (West Bengal) and lower Ganga (West Bengal). Based on previous reports, these sites are selected^{24,26}. Each section was again marked for four sampling points where the field setups were engaged making a total of 24 study sites which were within a radius of 1 km from the river bed. **Fig. S1** shows the sampling points with their specific coordinates. The experimental design of the study site follows similarly as in Majumdar et al²⁷ (**supplementary method**).

Soil-rice plant analysis for total and bioavailable arsenic and silicon. Total and bioavailable As concentrations were measured using wavelength dispersive X-ray fluorescence spectroscopy (WD-XRF, S8 Tiger, Bruker, Germany) and inductively-coupled plasma mass spectrophotometer (ICP-MS, Nexion 300X Parkin Elmer, US) respectively, according to the standardized method of Majumdar et al^{28,29}. Total Si in soil was measured using the WD-XRF method while bioavailable and plant Si concentrations were analyzed following the established method by Majumdar et al³⁰ and Mu et al³¹ (**supplementary method**). Standard reference materials (SRM) used in these analyses were SDC-1, JSD-3, MESS3 and NIST2711a and quality-checked data is provided in **Table S1**. Rice plants were analyzed for total As concentrations followed by the di-acid digestion-dilution method using ICP-MS and calibrated using the SRM NIST1568c.

Soil physico-chemical parameters and rice productivity. Oakton Waterproof PCS Testr35 and ORP Testr 10 were used as portable meters for pH and ORP testing in the fields whereas samples were analyzed for soil organic matter (SOM) and microbial biomass carbon (MBC) using the method described in Walkley and Black³². Soil textures were assessed considering the ASTM sieve plate method, standardized by Sarkar et al³³. To prove the effect of AWD unbiasedly, the study did not choose any specific rice cultivar during these trials and locally grown rice cultivars were considered. Each of the 800m² areas was assessed for rice grain yield and converted to hectare equivalent.

Economic profit validity and social acceptance. Rice yield per hectare under AWD and flooded practices were compared. The average of the minimum support price (MSP) of common and Grade A variety of paddy was used to estimate the price of paddy yield per hectare

and national reports by Ministry of Agriculture-Govt. of India, were used to estimate the cultivable areas for rice in the Gangetic valley. The total economic value per hectare yield along the study sites for pre-monsoonal and monsoonal using AWD technology is estimated for the entire Ganga valley.

Secondary analyses of As-Si association and plant physiology. Soil samples were analyzed for the possible oxy/hydroxygenic As and Si ions present in the soil during AWD or flooded conditions using ‘The Materials Project’ (an online database of NERSC Scientific Computing Center and Computational Research Division under Lawrence Berkeley National Library)^{30,34}. The pourbaix diagram, X-ray diffractogram and bubble plot analysis were generated based on the field data and database files together (**supplementary method**). Rice plant physiology was analyzed using field-emission scanning electron microscopy (FE-SEM, Carl Zeiss SUPRA 55VP) following the standardized method by Majumdar et al²⁹.

Soil microbial diversity assessment and next-generation sequencing. High-throughput sequencing was performed using isolated DNA samples from collected soils using a Qiagen DNeasy Powersoil kit (**supplementary method**). These finally retrieved raw data were implemented in making microbial diversity assessments, gene ontology, metabolic pathways identification using the Kyoto Encyclopedia of Genes and Genomes (KEGG), As-responsive gene activity and occurrences³⁵.

Statistical justifications and microbial data analysis. All soil analyses were performed in triplicates and tested for statistical significance at $p \leq 0.05$ using one-way analysis of variance (ANOVA). To confirm the factual significance level, Bonferroni Correction was performed as a post-hoc assessment of all the soil-plant data obtained³⁶. Experimental sites were 24, hence the considered variables were 24 to generate α -corrected p-value ≤ 0.00208 in Bonferroni Correction. For statistical analyses, graphical representations, microbial diversity assessments, gene ontology and KEGG analyses, GraphPad Prism 6, R Studio 2022, OriginPro 2018, SigmaPlot (ver. 12), ClustVis (ver 2.0 online), ShinyGO (ver 0.77 online), Cytoscape (ver. 3.8.2) and PAST (ver. 3.2.1) were used.

■ Results

Distribution of As-Si content in different geographic locations. Soil textures were found to be similar with minor variations in the composition of soil, silt and clay percentage of all 24 sites from four states and six Gangetic divisions (**Table S2**). Silt predominates at the middle to lower Gangetic belt, however, overall, the whole Ganga valley was found to be silty clay in

nature. A higher amount of clay in soil-sediment poses a barrier to As translocation to the rice plant³⁷. In **Fig. S2 (a-h)**, a contour plot of As-Si distribution related to their site-specific pH and ORP is shown. These graphs represent the mean data of 24 sites from two years together revealing the bulk elemental change with pH and ORP getting altered in the field. A lower ORP state can be found in anaerobic flooded conditions compared to the aerobic dry soils³⁸, supporting our findings as well. These lower ORP and comparatively high pH (7.6-8.9) resulted in the higher release of total As content from soil-associated minerals to the aqueous phase (**Fig. S2a-d**). High Si concentrations were found within higher ORP values that become predominant in soil under the AWD cycle as shown in **Fig. S2e-h**, irrespective of seasonal changes. Pre-monsoonal soil Si, although, gets more frequent AWD transition than the monsoonal soil Si, hence a difference of 5-20g kg⁻¹ was found between seasonal changes.

Fig. S3a-b shows the percentage area stacked plots of pre-monsoon and monsoon phases to understand the range of this As-Si distribution in individual Gangetic divisions (marked as divisions A-F). The upper Gangetic area showed minimum As concentrations in soil which comes from its lower As-contained groundwater²⁴ than the other states of the Ganga valley. The percentage plot clearly indicates the percent ratio of flooded soil As was greater than flooded soil Si and vice versa in AWD, during both seasons. Correlative plots of AWD and flooded soil physico-chemical parameters are emphasizing the relationship of bioavailable As-Si content to other influencing factors (**Fig. 1a-b**). This is a tri-parameter-based correlative analysis which indicates a positive or negative relationship based on color (red to violet), direction (upward or downward) and shape (slender to oval). Mean data of pH, ORP, OM, MBC and bioavailable As-Si were used for both pre-monsoon and monsoon during two consecutive years. ORP showed negative relation with As and positive relation with Si. During frequent ORP change, it contradicts soil pH values, as found in the pre-monsoon phase and AWD application¹² which in turn triggers the pulse release of available soil Si while entrapping the As content, reducing its bioavailability^{39,40}. Soil OM and MBC both displayed a positive effect on the soil Si availability while restricting the mobility of available As content. MBC can modulate OM which in turn contributes to the Si concentration³⁰ and found during the AWD applications.

The experiment used 24 sites and the average per hectare yield of paddy through AWD is 5774.02 kg/hectare compared to 2809 kg/hectare for the production for the country (**Fig. 2a**). Interestingly, the average per hectare water consumption consumed for paddy cultivation through AWD is one-fifth compared to the traditional methods used for paddy cultivation. The

per hectare average water consumption for paddy cultivation through ADW and FL, were 258437.5 litres, and 1316875 litres, respectively. If 10% of the Ganga valley used AWD technology for paddy cultivation the total annual water can be saved in the range of 1.354×10^{12} to 1.873×10^{12} litres. The water saving through AWD could act as a paradigm shift in a country like India where many of its states are water stress by 2030. This really helps to reduce the problem of water scarcity and helpful to achieve SDG 6 to clean water and sanitation and also economic benefits to the farmers²³.

Socio-economic validation and profitability analysis. The average per hectare revenue for AWD technology is estimated as INR 118367.4 (1442.14 USD) compared to INR 57584.5 (701.59 USD) for the country average, making farmer's per hectare average revenue increased by INR 60782.9 (740.55 USD) (**Fig. 2b**). The total increase in revenue for the farmers if 10% of the Ganga valley is used AWD is estimated as INR 82056.9 million (1000 million USD). The AWD technology is useful in terms of an increase in yield per hectare as a result an increase in total revenue. As a result, the farmers are gaining an increase in revenue, reducing the consumption of water, reducing the issues of food inequality and helping to achieve various sustainable goals through the use of ADW in paddy cultivation. **Table S5** and **S6** shows the details of economic profit from AWD implementation.

Secondary analyses and plant productivity in AWD and flooded cultivations. Soil As and Si were analyzed for the potential ionic species and their data-based molecular structures. Using 'The Materials Project', four molecular structures were simulated for As hydroxides and As-Si complexes (**Fig. S4a-b**). Field-obtained analyzed concentration range and other physical properties were provided as modelling input to generate these structures (SiAs_2 , Si_3As_4 , $\text{As}_3(\text{HO}_2)_5$ and As_3HO_6) emphasizing the stability of these compounds in the natural environments. Furthermore, a pourbaix diagram was generated (**Fig. S4c**) using the concentration range in molar values⁴¹. The generated graph suggests the predominant species of As and Si independently within a pH range of 6.4 to 8.7 and ORP of -185 to +290 mV. Predominant ionic species of As and Si were found to be H_2AsO_4^- , HAsO_4^{2-} , HAsO_2 , and SiO_2 within this range, as marked by the red-colored box in the figure. The generated structures were further validated using the 'The Materials Project' database of elemental confirmatory XRD assessments (**Fig. S5a**). The XRD graphs were generated by comparing our experimental data to the online database of these four compounds mentioned. The graph shows single peak lines and a brown-colored reference line to show the exact match of these compounds, indicating the acceptability of these structures as valid naturally present compounds⁴². Bubble plots of

soil As and Si compounds were obtained under AWD and flooded conditions in both seasons where OM was considered as the influencing factor. It appears that the AWD has a marked effect on As capture and Si release¹⁵ (**Fig. S5b**).

Plant internal anatomy and ultra-structure of vascular systems were observed using FE-SEM from 24 study sites (six Gangetic divisions) under both AWD and flooded and presented in **Fig. S6**. Xylem (Xy) and Phloem (Ph) are marked in the figures to elucidate the prominent difference between the vasculature of AWD-grown plants and flooded-grown plants^{30,43}. Improved rice internal anatomy is due to the fact of higher Si and lower As accumulation which directly correlates with the bioavailable amount of soil As and Si under AWD and flooded field conditions (**Fig. S7a-f**). A distinct and consistent pattern of As bioavailability was observed in pre-monsoonal AWD for all 24 sites while the highest plant available As was recorded in flooded soils during monsoonal season. However, higher plant-available Si was observed in pre-monsoonal AWD soils. Higher Si and lower As increase the rice plant cellulose synthase gene expressions for CESA3 and CESA4, resulting in higher cellulose production and therefore better structural rigidity⁴⁴. These combined effects of bioavailability and structural betterments proved the plant's positive response towards AWD, resulted in greater rice grain productivity in all Gangetic divisions. Rice productivity was measured in kg ha⁻¹ and shown in **Fig. S7g-l**. The data trend clearly reflects the effectivity of AWD practice over the flooded condition with greater statistical significance as marked in the figure. The highest range of rice yield was assessed in lower-middle and lower Gangetic divisions under the West Bengal district.

Microbial diversity and KEGG metabolic pathway assessments. Soil microbial communities are sensitive to any change in soil status and agronomic practices^{16,19} which triggers their gene expressions, metabolic activities, and tolerance towards abiotic stresses. Soil microbial diversity was assessed using a next-generation sequencing technique which allows us to find variance in the microbial predominance in soil under different circumstances²⁷. **Fig. S8** shows the predominant microbial classes under AWD and flooded conditions with seasonal changes. Among the 94-107 classes, 74 classes are presented in this color-coded heatmap which was present in all soil samples but differed in their number and degree of occurrence. It is evident that most of these microbial communities were well established under the dry-wet condition of the pre-monsoonal phase followed by the monsoonal dry-wet cultivation. Flooded conditions in both seasons were lower in harbouring microbial diversity. How AWD soil might promote microbial growth, communities and their metabolism has been suggested in some earlier studies^{27,45}. Microbial diversity can be estimated^{27,45} by analyzing their alpha and beta

diversity indices as given in **Table S3**. Mean values are presented in this table for all six Gangetic divisions for three different species richness indices- Shannon, Simpson and Chao1. These indices show that a higher species richness and microbial diversity were present in AWD soils compared to the flooded soils and pre-monsoonal phase had a greater richness overall than the monsoonal richness.

Irrespective of geographical locations, soil microbial metabolism showed greater expressions and activities when AWD was implemented. **Fig. 3a-b** shows a color-coded heat map of the analyzed and counted KEGG modules from all 24 sites of the six Gangetic divisions. A total of 164 metabolism-related pathways are presented in two different graphs for better visibility. These KEGG modules are presented with their KEGG numbers which can further tell us the details of the pathway compositions and counter-parts. The color distribution clearly indicates the dominance of pre-monsoonal dry-wet soil had a greater impact on the microbial communities which triggered most of the metabolic pathways to be active and expressed⁴⁶. By way of illustration, 26 pathways from these KEGG modules are presented in **Fig. S9**, which are essential for the viability and stability of microbial growth and metabolism. Purine and pyrimidine metabolism; biosynthesis and degradation of fatty acids; glycolysis/gluconeogenesis; metabolisms of amino acids; carbohydrate metabolisms including pentose sugar and galactose; metabolic activities of Krebs's cycle; synthesis of other essential bio-molecules including lipopolysaccharides, pantothenate, co-enzyme A, and folic acid; metabolism of other essential bio-molecules including nicotinamide, lipoic acid, thiamine, riboflavin, and biotin are presented in a database-dependent pathway marking manner⁴⁷. The red color indicates active enzymes, precursors, and their metabolic products within a pathway chart from our given input. Several pathways were found to be less active under flooded soil, as shown also in **Fig. 3a-b**. Greater occurrence and activities of microbial metabolic activities under AWD likely contribute to prove healthier soil compared to flooded soil^{20,46}.

Microbial gene ontology and arsenic resistance. Gene ontology is used to understand which gene clusters and their representative molecular functions are active under AWD and flooded soils and getting altered with seasonal changes^{27,46}. Again, an average data of gene ontology obtained from next-generation sequencing has been presented in **Fig. 4a-b**. High frequency and low frequency genes are counted at the Illumina platform. It was evident that the highest gene ontological encounter in dry-wet soils compared to the flooded soils was found in both high-frequency and low-frequency genes. Similarly, another color-coded heat map distribution of these high-frequency and low-frequency gene ontological terms is presented in **Fig. S10**.

These heat maps show the intensity of these high-frequency and low-frequency gene ontological terms for AWD and flooded soil microbiomes. These combined ontology assessments prove the efficiency of microbial genes and their respective activities which comes under three different categories- Biological processes (GO:0008150), Cellular components (GO:0005575) and Molecular functions (GO:0003674)⁴⁸.

This further encouraged us to define the arsenic-responsive genes and their relationship with microbial diversity distribution under these altered soil statuses⁴⁹. Some of these genes can govern the microbial ability to modulate total and bioavailable As concentration in soil depending on the environmental factors. From the obtained data of gene-specific high throughput sequencing and microbial distribution, a networking analysis is presented in **Fig. S11a-d**. Genes including *arsA*, *arsB*, *arsC*, *arsD*, *arsH*, *arsM*, *arsR*, *arsT*, *aioA/aoxB*, *aioE*, *aioS/aoxS*, *aioR/aoxR*, *aioX/aoxX*, *arrA*, *arrB*, *pstA_1*, *pstB*, *pstC_1*, *pstS1*, *glpF_1*, *ctaF*, and *pgpA/ltpgpA* were more connected to an extensive microbial genus network in AWD soils both under pre-monsoon and monsoonal changes compared to the flooded soil microbial genera. Cytoscape ‘String’ network has been implemented here which allows to visualize of gene-translated protein PDB (protein database) structures from these networks⁵⁰. A number of microbial genera connected to each of these genes within these networks strongly suggests the involvement of microbial diversity expressing As-responsive genes profoundly under AWD which results in the As transformation and its reduced mobilization into the soil system.

The arsenic response and tolerance in microbes are one of the primitive cellular activities⁵¹ which is also related to other cellular functions. In another set of analysis, *arrB*, *aioA*, *arrA*, *arsR*, *arsB*, *arsC*, *arsA*, *arsR*, *arsD*, *arsH*, *aioE*, *arsT*, *arrB*, *pstS*, *pstC*, *pstB*, *pstA*, *pgpA*, *arsP*, *arsM*, *aioX*, *aioS*, *aioR*, *aioA*, *aoxX*, *aoxS*, *aoxR*, *aoxB*, *ltpgpA*, and *aoxB* genes are presented with their corresponding cellular and physiological activity profiling in **Fig. 5**. Physiological responses including protein functions are mentioned in **Fig. 5** which has some direct or indirect link to As metabolism and/or tolerance. The color-coded fold enrichment values and types of parameters suggest that phosphate transporters, arsenical transporters, and arsenical efflux proteins are more expressed under AWD soil microbes in both pre-monsoonal and monsoonal phases while a higher expressions of phosphate transporter, arsenic transporter proteins, cell membrane proteins and other membrane stabilizing proteins under flooded soil microbes in both the seasons. This indicates additional cell-stressing activities are being managed by the microbial cells by producing and expressing different membrane-stabilizing proteins and bio-molecules under flooded conditions⁵². Further, a networking analysis of these genes and their

products or their molecular functions is presented in **Fig. S12**. Various secondary ion transporters, phosphate transporters, arsenical transporters proteins, arsenic efflux proteins, anion and cation transporters, As-resistance biomolecules, redox-active centers and membrane-associated proteins are interconnected showing the complex inter-relationship of these biomolecules depending on their degree of expression under AWD and flooded conditions with seasonal changes⁵³. A gradient of network complexity was observed in the following sequence- pre-monsoon AWD, pre-monsoon flooded, monsoon AWD and monsoon flooded microbiome. These assessments are generated using the GO terms obtained from experimental data, merging with the online database using ShinyGO software to identify the inter-connectivity among these genes and their products⁵⁴.

Discussions

The project started with a motto of promoting AWD in the Indian sub-continent and gradually to the rest of the world as much as possible to make common people aware of the scarcity of the freshwater that we need for crop cultivation. Instead of keeping the field waterlogged for the whole time, requiring an average stagnant depth of 14-17cm, the AWD practice needs only 3-5cm waterlogging for 2 days and a drying phase reducing water usage by 52-75% depending on the soil texture and climatic conditions^{55,56}. This study also minimized unnecessary groundwater consumption emphasizing **SDG 6** (Clean water and sanitation) and **SDG 12** (Responsible consumption and production) and their promotion to the farmers for future practice. **Table S4** shows the details of water conservation by AWD practice. Excessive extraction of groundwater also increases the risk of As dissolution from the deep aquifer to the groundwater⁵⁷, making it contaminated for direct use or for rice cultivation. Also, in the upper Gangetic division, where the annual average temperature is lower than the middle to lower Gangetic divisions, requirements for water were less due to lesser evapotranspiration⁵⁶. This proves again, AWD practice can ‘save water’ by promoting SDG 6 at a large scale.

SDG 3 (Good health and wellbeing) and **SDG 9** (Industry, innovation, and infrastructure) promote issues related to soil management and their effects on human health. Soil As distribution is a global threat which gets spread through As-contaminated food stuff, primarily rice and rice-based foods^{58,59}. More toxic species of As to the rice plant and its consumers, is generated in the flooded field than AWD soils⁶⁰. When soil As bioavailability gets minimized, and rice quality improves, the consumers’ health improves and is risk-free^{61,62}. While reducing As availability, AWD, on the contrary, influence the plant-available Si to be more labile due to the frequent alterations of pH, redox potentials and OM content^{12,39}. This agronomic practice

can certainly improve the well-being of our consumers if a proper infrastructure for implementing AWD can be developed by joint ventures of scientists, engineers and policymakers. **SDG 2** (Zero hunger) urges global researchers and policymakers to develop such practices which will promote higher food production minimizing the hunger ratio and malnutrition status globally^{63,64}. Our earlier long-term field experiment has proved the efficacy of AWD in a greater nutritional enrichment in rice grain with higher productivity^{27,58} which further has been experimentally established in this project. In **Fig. 2**, rice productivity has been presented indicating the direct effect of AWD on rice grain yield compared to conventional waterlogged cultivation. AWD triggers the development of higher panicle numbers and grain fillings after the inflorescence stage⁶⁵. Better ultra-structure rigidity in AWD-grown plants also indicates a healthier state than the flooded-grown plants (**Fig. S6**). The results establish the fact that irrespective of geographic locations and seasonal changes, AWD can deliver high rice productivity, hence minimizing the crisis of food supply to the market, especially in arid countries¹. With higher productivity, AWD also ensures greater economic benefit to the farmers and a cost-effective cultivation process (**Fig. S7**). This promotes **SDG 8** (Decent work and economic growth) and appeals to the government or non-government organizations to popularize the AWD practice in crop cultivation which can generate considerable revenue compared to the conventional mode of rice cultivation.

After analyzing soil microbiomes for their diversity depending on the water management practices, and seasonal variations, it becomes evident that AWD can induce microbial growth and species richness better than the flooded field conditions (**Table S3**), further supported by the expression of the metabolic pathways obtained by KEGG analysis (**Fig. 3** and **Fig. S9**). Obtained results of KEGG pathway matching of essential metabolic activities from AWD microbiome shows similar results reported in other studies^{45,46,66}. AWD implementation can certainly influence microbial molecular responses by triggering their gene expressions^{49,51} as found in the GO analysis from altered water regimes and seasonal changes (**Fig. 4** and **Fig. S10**). This can be understood by observing the data presented in Fig. SA where greater microbial genera participated in the As-resistance and phosphate transportation, cell membrane protein function and stability management, other anionic and cationic transfer activities⁴⁸. Microbial cellular responses and physiological traits based on these molecular expressions are triggered under AWD (**Fig. S11** and **Fig. S12**). Under flooded soil, most of the aerobic microbes become dormant or eliminated and the continued prevalence of reducing environment hinders some other soil meso-biota⁶⁷. To promote soil health and soil quality, soil meso- and

microbiota must be conserved (**SDG 15**, Life on land) which can be achieved by implementing AWD practice. A complex cycle of biotic-abiotic balance is well preserved and thrived under AWD practice than the flooded conditions^{68,69}.

Acknowledgements

The authors are thankful to IISER-K for providing infrastructure as well as core facilities (XRF DST-FIST laboratory, FE-SEM Laboratory). AM is thankful to the IISER Kolkata and SERB-Govt. of India for Postdoctoral fellowship and research grant (File no- PDF/2022/001418/LS) for providing fellowship. Authors are also thankful to Genotypic Technology [P] Ltd., Bangalore, and Xcelris Labs Limited, Gujrat, India for the genomics analysis. The authors are also thankful to the junior students from each of these study areas who helped with sample collection and collected the basic information required.

Author credit statement

AM- conceptualization and project development, partial sampling and field management, major lab experiments, supervision, write-up and revisions; MKU- partial sampling and field management, partial write-up; BG- WD-XRF analysis; DM and SS- Metagenomics and high throughput sequencing analysis, partial write-up; BKT- economic value assessments and partial write-up; PY- partial graphics and write-up; KS- FE-SEM analysis; AKS- metagenomics and high throughput sequencing analysis; MKJ- supervision; MB- Metagenomics and high throughput sequencing analysis and supervision; MT- Metagenomics and high throughput sequencing analysis and supervision; TRC- partial write-up and supervision

Declaration for conflict of interest

The authors declare there is no conflict of interest known for this project and manuscript.

References

- (1) Moulick, D.; Ghosh, D.; Skalicky, M.; Gharde, Y.; Mazumder, M. K.; Choudhury, S.; Biswas, J. K.; Santra, S. C.; Brestic, M.; Vachova, P. Interrelationship among Rice Grain Arsenic, Micronutrients Content and Grain Quality Attributes: An Investigation from Genotype× Environment Perspective. *Front. Environ. Sci.* **2022**, 180.
- (2) Upadhyay, M. K.; Majumdar, A.; Barla, A.; Bose, S.; Srivastava, S. An Assessment of Arsenic Hazard in Groundwater–Soil–Rice System in Two Villages of Nadia District, West Bengal, India. *Environ. Geochem. Health* **2019**, 41, 2381–2395.
- (3) Neumann, R. B.; Pracht, L. E.; Polizzotto, M. L.; Badruzzaman, A. B. M.; Ali, M. A. Sealing Rice Field Boundaries in Bangladesh: A Pilot Study Demonstrating

- Reductions in Water Use, Arsenic Loading to Field Soils, and Methane Emissions from Irrigation Water. *Environ. Sci. Technol.* **2014**, *48* (16), 9632–9640.
- (4) Shaji, E.; Santosh, M.; Sarath, K. V; Prakash, P.; Deepchand, V.; Divya, B. V. Arsenic Contamination of Groundwater: A Global Synopsis with Focus on the Indian Peninsula. *Geosci. Front.* **2021**, *12* (3), 101079.
 - (5) Bundschuh, J.; Armienta, M. A.; Morales-Simfors, N.; Alam, M. A.; López, D. L.; Delgado Quezada, V.; Dietrich, S.; Schneider, J.; Tapia, J.; Sracek, O. Arsenic in Latin America: New Findings on Source, Mobilization and Mobility in Human Environments in 20 Countries Based on Decadal Research 2010-2020. *Crit. Rev. Environ. Sci. Technol.* **2021**, *51* (16), 1727–1865.
 - (6) Singh, S.; Rajput, V. D.; Upadhyay, S. K.; Minkina, T. Arsenic Contamination in Rice Agro-Ecosystems: Mitigation Strategies for Safer Crop Production. *J. Plant Growth Regul.* **2022**, 1–12.
 - (7) Harine, I. J.; Islam, M. R.; Hossain, M.; Afroz, H.; Jahan, R.; Siddique, A. B.; Uddin, S.; Hossain, M. A.; Alamri, S.; Siddiqui, M. H.; Henry, R. J. Arsenic Accumulation in Rice Grain as Influenced by Water Management: Human Health Risk Assessment. *Agronomy* **2021**, *11* (9), 1–13. <https://doi.org/10.3390/agronomy11091741>.
 - (8) Abedin, J.; Cresser, M. S.; Meharg, A. A.; Feldmann, J.; Cotter-Howells, J. Arsenic Accumulation and Metabolism in Rice (*Oryza Sativa* L.). *Environ. Sci. Technol.* **2002**, *36* (5), 962–968. <https://doi.org/10.1021/es0101678>.
 - (9) Yuan, S.; Linqvist, B. A.; Wilson, L. T.; Cassman, K. G.; Stuart, A. M.; Pede, V.; Miro, B.; Saito, K.; Agustiani, N.; Aristya, V. E. Sustainable Intensification for a Larger Global Rice Bowl. *Nat. Commun.* **2021**, *12* (1), 7163.
 - (10) Raturi, G.; Sharma, Y.; Rana, V.; Thakral, V.; Myaka, B.; Salvi, P.; Singh, M.; Dhar, H.; Deshmukh, R. Exploration of Silicate Solubilizing Bacteria for Sustainable Agriculture and Silicon Biogeochemical Cycle. *Plant Physiol. Biochem.* **2021**, *166*, 827–838.
 - (11) Liu, W.-J.; McGrath, S. P.; Zhao, F.-J. Silicon Has Opposite Effects on the Accumulation of Inorganic and Methylated Arsenic Species in Rice. *Plant Soil* **2014**, *376*, 423–431.
 - (12) Kabata-Pendias, A. *Trace Elements in Soils and Plants*; CRC press, 2010.
 - (13) Vories, E.; Rhine, M.; Straatmann, Z. Investigating Irrigation Scheduling for Rice Using Variable Rate Irrigation. *Agric. Water Manag.* **2017**, *179*, 314–323.
 - (14) Li, C.; Carrijo, D. R.; Nakayama, Y.; Linqvist, B. A.; Green, P. G.; Parikh, S. J. Impact of Alternate Wetting and Drying Irrigation on Arsenic Uptake and Speciation in Flooded Rice Systems. *Agric. Ecosyst. Environ.* **2019**, *272*, 188–198.
 - (15) Rokonzaman, M. D.; Ye, Z.; Wu, C.; Li, W. Arsenic Accumulation in Rice: Alternative Irrigation Regimes Produce Rice Safe from Arsenic Contamination. *Environ. Pollut.* **2022**, *310*, 119829.
 - (16) Cao, X.; Zhang, J.; Yu, Y.; Ma, Q.; Kong, Y.; Pan, W.; Wu, L.; Jin, Q. Alternate Wetting–Drying Enhances Soil Nitrogen Availability by Altering Organic Nitrogen Partitioning in Rice-Microbe System. *Geoderma* **2022**, *424*, 115993.

- (17) Sekar, I.; Pal, S. Rice and Wheat Crop Productivity in the Indo-Gangetic Plains of India: Changing Pattern of Growth and Future Strategies. *Indian J. Agric. Econ.* **2012**, *67* (902-2016–67295).
- (18) Ahamed, S.; Sengupta, M. K.; Mukherjee, A.; Hossain, M. A.; Das, B.; Nayak, B.; Pal, A.; Mukherjee, S. C.; Pati, S.; Dutta, R. N. Arsenic Groundwater Contamination and Its Health Effects in the State of Uttar Pradesh (UP) in Upper and Middle Ganga Plain, India: A Severe Danger. *Sci. Total Environ.* **2006**, *370* (2–3), 310–322.
- (19) Zheng, T.; Deng, Y.; Wang, Y.; Jiang, H.; O’Loughlin, E. J.; Flynn, T. M.; Gan, Y.; Ma, T. Seasonal Microbial Variation Accounts for Arsenic Dynamics in Shallow Alluvial Aquifer Systems. *J. Hazard. Mater.* **2019**, *367*, 109–119.
- (20) Jeong, S.; Hong, J. K.; Jho, E. H.; Nam, K. Interaction among Soil Physicochemical Properties, Bacterial Community Structure, and Arsenic Contamination: Clay-Induced Change in Long-Term Arsenic Contaminated Soils. *J. Hazard. Mater.* **2019**, *378*, 120729.
- (21) Jia, Y.; Huang, H.; Chen, Z.; Zhu, Y.-G. Arsenic Uptake by Rice Is Influenced by Microbe-Mediated Arsenic Redox Changes in the Rhizosphere. *Environ. Sci. Technol.* **2014**, *48* (2), 1001–1007.
- (22) Reid, M. C.; Maillard, J.; Bagnoud, A.; Falquet, L.; Le Vo, P.; Bernier-Latmani, R. Arsenic Methylation Dynamics in a Rice Paddy Soil Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **2017**, *51* (18), 10546–10554.
- (23) Lal, R.; Bouma, J.; Brevik, E.; Dawson, L.; Field, D. J.; Glaser, B.; Hatano, R.; Hartemink, A. E.; Kosaki, T.; Lascelles, B. Soils and Sustainable Development Goals of the United Nations: An International Union of Soil Sciences Perspective. *Geoderma Reg.* **2021**, *25*, e00398.
- (24) Singh, M.; Singh, A. K.; Swati; Srivastava, N.; Singh, S.; Chowdhary, A. K. Arsenic Mobility in Fluvial Environment of the Ganga Plain, Northern India. *Environ. Earth Sci.* **2010**, *59*, 1703–1715.
- (25) Upadhyay, M. K.; Majumdar, A.; Suresh Kumar, J.; Srivastava, S. Arsenic in Rice Agro-Ecosystem: Solutions for Safe and Sustainable Rice Production. *Front. Sustain. Food Syst.* **2020**, *4* (May). <https://doi.org/10.3389/fsufs.2020.00053>.
- (26) Tyagi, N.; Upadhyay, M. K.; Majumdar, A.; Pathak, S. K.; Giri, B.; Jaiswal, M. K.; Srivastava, S. An Assessment of Various Potentially Toxic Elements and Associated Health Risks in Agricultural Soil along the Middle Gangetic Basin, India. *Chemosphere* **2022**, *300*, 134433.
- (27) Majumdar, A.; Dubey, P. K.; Giri, B.; Moulick, D.; Srivastava, A. K.; Roychowdhury, T.; Bose, S.; Jaiswal, M. K. Combined Effects of Dry-Wet Irrigation, Redox Changes and Microbial Diversity on Soil Nutrient Bioavailability in the Rice Field. *Soil Tillage Res.* **2023**, *232*, 105752.
- (28) Majumdar, A.; Upadhyay, M. K.; Giri, B.; Karwadiya, J.; Bose, S.; Jaiswal, M. K. Iron Oxide Doped Rice Biochar Reduces Soil-Plant Arsenic Stress, Improves Nutrient Values: An Amendment towards Sustainable Development Goals. *Chemosphere* **2023**, *312*, 137117.
- (29) Majumdar, A.; Upadhyay, M. K.; Ojha, M.; Afsal, F.; Giri, B.; Srivastava, S.; Bose, S.

- Enhanced Phytoremediation of Metal (Loid) s via Spiked ZVI Nanoparticles: An Urban Clean-up Strategy with Ornamental Plants. *Chemosphere* **2022**, *288*, 132588.
- (30) Majumdar, A.; Upadhyay, M. K.; Kumar, J. S.; Barla, A.; Srivastava, S.; Jaiswal, M. K.; Bose, S. Ultra-Structure Alteration via Enhanced Silicon Uptake in Arsenic Stressed Rice Cultivars under Intermittent Irrigation Practices in Bengal Delta Basin. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 770–779.
- (31) Mu, J.; Hu, Z.; Huang, L.; Xie, Z.; Holm, P. E. Preparation of a Silicon-Iron Amendment from Acid-Extracted Copper Tailings for Remediating Multi-Metal-Contaminated Soils. *Environ. Pollut.* **2020**, *257*, 113565.
- (32) Walkley, A.; Black, I. A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37* (1), 29–38.
- (33) Sarkar, S. R.; Majumdar, A.; Barla, A.; Pradhan, N.; Singh, S.; Ojha, N.; Bose, S. A Conjugative Study of Typha Latifolia for Expunge of Phyto-Available Heavy Metals in Fly Ash Ameliorated Soil. *Geoderma* **2017**, *305*, 354–362.
- (34) Jain, A.; Montoya, J.; Dwaraknath, S.; Zimmermann, N. E. R.; Dagdelen, J.; Horton, M.; Huck, P.; Winston, D.; Cholia, S.; Ong, S. P. The Materials Project: Accelerating Materials Design through Theory-Driven Data and Tools. *Handb. Mater. Model. Methods Theory Model.* **2020**, 1751–1784.
- (35) Kakui, K.; Fleming, J. F.; Mori, M.; Fujiwara, Y.; Arakawa, K. Comprehensive Transcriptome Sequencing of Tanaidacea with Proteomic Evidences for Their Silk. *Genome Biol. Evol.* **2021**, *13* (12), evab281.
- (36) Li, W.; Liu, Q.; Xie, L.; Yin, C. Interspecific Plant-Plant Interactions Increase the Soil Microbial Network Stability, Shift Keystone Microbial Taxa, and Enhance Their Functions in Mixed Stands. *For. Ecol. Manage.* **2023**, *533*, 120851.
- (37) Liu, W. J.; Zhu, Y. G.; Hu, Y.; Williams, P. N.; Gault, A. G.; Meharg, A. A.; Charnock, J. M.; Smith, F. A. Arsenic Sequestration in Iron Plaque, Its Accumulation and Speciation in Mature Rice Plants (*Oryza Sativa* L.). *Environ. Sci. Technol.* **2006**, *40* (18), 5730–5736. <https://doi.org/10.1021/es060800v>.
- (38) Khan, S. U.; Hooda, P. S.; Blackwell, M. S. A.; Busquets, R. Effects of Drying and Simulated Flooding on Soil Phosphorus Dynamics from Two Contrasting UK Grassland Soils. *Eur. J. Soil Sci.* **2022**, *73* (1), e13196.
- (39) Savant, N. K.; Snyder, G. H.; Datnoff, L. E. Silicon Management and Sustainable Rice Production. *Adv. Agron.* **1996**, *58*, 151–199.
- (40) Khan, I.; Awan, S. A.; Rizwan, M.; Ali, S.; Hassan, M. J.; Brestic, M.; Zhang, X.; Huang, L. Effects of Silicon on Heavy Metal Uptake at the Soil-Plant Interphase: A Review. *Ecotoxicol. Environ. Saf.* **2021**, *222*, 112510.
- (41) Wang, Z.; Guo, X.; Montoya, J.; Nørskov, J. K. Predicting Aqueous Stability of Solid with Computed Pourbaix Diagram Using SCAN Functional. *npj Comput. Mater.* **2020**, *6* (1), 160.
- (42) Lee, B. Do; Lee, J.-W.; Park, W. B.; Park, J.; Cho, M.-Y.; Pal Singh, S.; Pyo, M.; Sohn, K.-S. Powder X-Ray Diffraction Pattern Is All You Need for Machine-Learning-Based Symmetry Identification and Property Prediction. *Adv. Intell. Syst.* **2022**, *4* (7),

2200042.

- (43) Concenço, G.; de Brito, G. G.; Fagundes, P. R. R.; Scivittaro, W. B.; Parfitt, J. M. B.; Melo, T. S.; da SILVA, L. B. X.; Deuner, S. Contribution for Improvement of Rice Breeding Programs through Morphoanatomical Traits. **2021**.
- (44) Srivastava, A. K.; Pandey, M.; Ghate, T.; Kumar, V.; Upadhyay, M. K.; Majumdar, A.; Sanjukta, A. K.; Agrawal, A. K.; Bose, S.; Srivastava, S. Chemical Intervention for Enhancing Growth and Reducing Grain Arsenic Accumulation in Rice. *Environ. Pollut.* **2021**, *276*, 116719.
- (45) Hester, E. R.; Vaksmaa, A.; Valè, G.; Monaco, S.; Jetten, M. S. M.; Lüke, C. Effect of Water Management on Microbial Diversity and Composition in an Italian Rice Field System. *FEMS Microbiol. Ecol.* **2022**, *98* (3), fiac018.
- (46) Wu, J.; Ma, C.; Li, F. Microbial Community Structure and Function in Paddy Soil as Affected by Water-Saving Irrigation Mode. *Eur. J. Soil Biol.* **2022**, *113*, 103450.
- (47) Kanehisa, M.; Furumichi, M.; Sato, Y.; Ishiguro-Watanabe, M.; Tanabe, M. KEGG: Integrating Viruses and Cellular Organisms. *Nucleic Acids Res.* **2021**, *49* (D1), D545–D551.
- (48) Behera, B. K.; Chakraborty, H. J.; Patra, B.; Rout, A. K.; Dehury, B.; Das, B. K.; Sarkar, D. J.; Parida, P. K.; Raman, R. K.; Rao, A. R. Metagenomic Analysis Reveals Bacterial and Fungal Diversity and Their Bioremediation Potential from Sediments of River Ganga and Yamuna in India. *Front. Microbiol.* **2020**, *11*, 556136.
- (49) Yuan, C.; Qiao, J.; Li, F.; Zhang, X.; Du, Y.; Hu, M.; Sun, W. Community Dynamics of As (V)-Reducing and As (III)-Oxidizing Genes during a Wet–Dry Cycle in Paddy Soil Amended with Organic Matter, Gypsum, or Iron Oxide. *J. Hazard. Mater.* **2020**, *393*, 122485.
- (50) Doncheva, N. T.; Morris, J. H.; Gorodkin, J.; Jensen, L. J. Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *J. Proteome Res.* **2018**, *18* (2), 623–632.
- (51) Ben Fekih, I.; Zhang, C.; Li, Y. P.; Zhao, Y.; Alwathnani, H. A.; Saquib, Q.; Rensing, C.; Cervantes, C. Distribution of Arsenic Resistance Genes in Prokaryotes. *Front. Microbiol.* **2018**, *9*, 2473.
- (52) Randle-Boggis, R. J.; Ashton, P. D.; Helgason, T. Increasing Flooding Frequency Alters Soil Microbial Communities and Functions under Laboratory Conditions. *Microbiologyopen* **2018**, *7* (1), e00548.
- (53) Anawar, H. M.; Rengel, Z.; Damon, P.; Tibbett, M. Arsenic-Phosphorus Interactions in the Soil-Plant-Microbe System: Dynamics of Uptake, Suppression and Toxicity to Plants. *Environ. Pollut.* **2018**, *233*, 1003–1012.
- (54) Ge, S. X.; Jung, D.; Yao, R. ShinyGO: A Graphical Gene-Set Enrichment Tool for Animals and Plants. *Bioinformatics* **2020**, *36* (8), 2628–2629.
- (55) Ishfaq, M.; Farooq, M.; Zulfiqar, U.; Hussain, S.; Akbar, N.; Nawaz, A.; Anjum, S. A. Alternate Wetting and Drying: A Water-Saving and Ecofriendly Rice Production System. *Agric. Water Manag.* **2020**, *241*, 106363.
- (56) Pande, C. B.; Moharir, K. N.; Varade, A. Water Conservation Structure as an

- Unconventional Method for Improving Sustainable Use of Irrigation Water for Soybean Crop under Rainfed Climate Condition. In *Climate change impacts on natural resources, ecosystems and agricultural systems*; Springer, 2023; pp 629–641.
- (57) Saftner, D. M.; Bacon, S. N.; Arienzo, M. M.; Robtoy, E.; Schlauch, K.; Neveux, I.; Grzymalski, J. J.; Carbone, M. Predictions of Arsenic in Domestic Well Water Sourced from Alluvial Aquifers of the Western Great Basin, USA. *Environ. Sci. Technol.* **2023**, *57* (8), 3124–3133.
- (58) Chowdhury, N. R.; Das, A.; Joardar, M.; Mridha, D.; De, A.; Majumder, S.; Mandal, J.; Majumdar, A.; Roychowdhury, T. Distribution of Arsenic in Rice Grain from West Bengal, India: Its Relevance to Geographical Origin, Variety, Cultivars and Cultivation Season. In *Global Arsenic Hazard: Ecotoxicology and Remediation*; Springer, 2022; pp 509–531.
- (59) Zhao, F. J.; Zhu, Y. G.; Meharg, A. A. Methylated Arsenic Species in Rice: Geographical Variation, Origin, and Uptake Mechanisms. *Environ. Sci. Technol.* **2013**, *47* (9), 3957–3966. <https://doi.org/10.1021/es304295n>.
- (60) Chen, C.; Yu, Y.; Wang, Y.; Gao, A.; Yang, B.; Tang, Z.; Zhao, F.-J. Reduction of Dimethylarsenate to Highly Toxic Dimethylarsenite in Paddy Soil and Rice Plants. *Environ. Sci. Technol.* **2022**, *57* (1), 822–830.
- (61) Joardar, M.; Mukherjee, P.; Das, A.; Mridha, D.; De, A.; Chowdhury, N. R.; Majumder, S.; Ghosh, S.; Das, J.; Alam, M. R. Different Levels of Arsenic Exposure through Cooked Rice and Its Associated Benefit-Risk Assessment from Rural and Urban Populations of West Bengal, India: A Probabilistic Approach with Sensitivity Analysis. *Environ. Sci. Pollut. Res.* **2023**, *30* (27), 70950–70973.
- (62) Nunes, L. M.; Li, G.; Chen, W.-Q.; Meharg, A. A.; O’Connor, P.; Zhu, Y.-G. Embedded Health Risk from Arsenic in Globally Traded Rice. *Environ. Sci. Technol.* **2022**, *56* (10), 6415–6425.
- (63) Blesh, J.; Hoey, L.; Jones, A. D.; Friedmann, H.; Perfecto, I. Development Pathways toward “Zero Hunger.” *World Dev.* **2019**, *118*, 1–14.
- (64) Kumar, K. A.; Rajitha, G. Alternate Wetting and Drying (AWD) Irrigation—a Smart Water Saving Technology for Rice: A Review. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8* (3), 2561–2571.
- (65) Shrivastava, A.; Barla, A.; Majumdar, A.; Singh, S.; Bose, S. Arsenic Mitigation in Rice Grain Loading via Alternative Irrigation by Proposed Water Management Practices. *Chemosphere* **2020**, *238*, 124988.
- (66) Min, D.; Cheng, L.; Liu, D.-F.; Liu, J.-Q.; Li, W.-W.; Yu, H.-Q. Single Strain-Triggered Biogeochemical Cycle of Arsenic. *Environ. Sci. Technol.* **2022**, *56* (22), 16410–16418.
- (67) Bambaradeniya, C. N. B.; Amerasinghe, F. P. Biodiversity Associated with the Rice Field Agroecosystem in Asian Countries: A Brief Review. **2004**.
- (68) Afsal, F.; Majumdar, A.; Kumar, J. S.; Bose, S. Microbial Inoculation to Alleviate the Metal Toxicity in Crop Plants and Subsequent Growth Promotion. *Sustain. Solut. Elem. Defic. Excess Crop Plants* **2020**, 451–479. https://doi.org/10.1007/978-981-15-8636-1_17.

- (69) Plum, N. Terrestrial Invertebrates in Flooded Grassland: A Literature Review. *Wetlands* **2005**, 25 (3), 721–737.

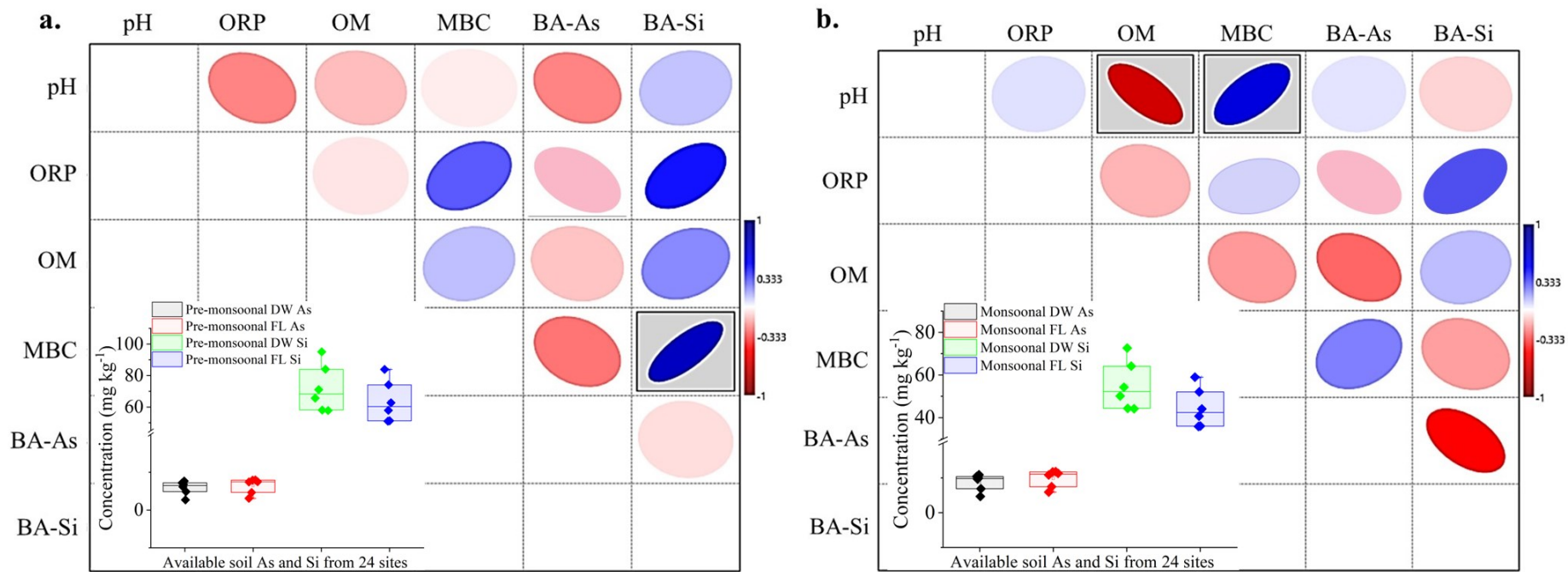


Fig. 1. Irrigation-influenced bioavailable As and Si concentration in all 24 sites and correlation with soil properties. The concentration range of soil As and Si has been presented in a box plot. Both pre-monsoonal (a) and monsoonal (b) results are presented with physico-chemical parameter-dependent correlative plots.

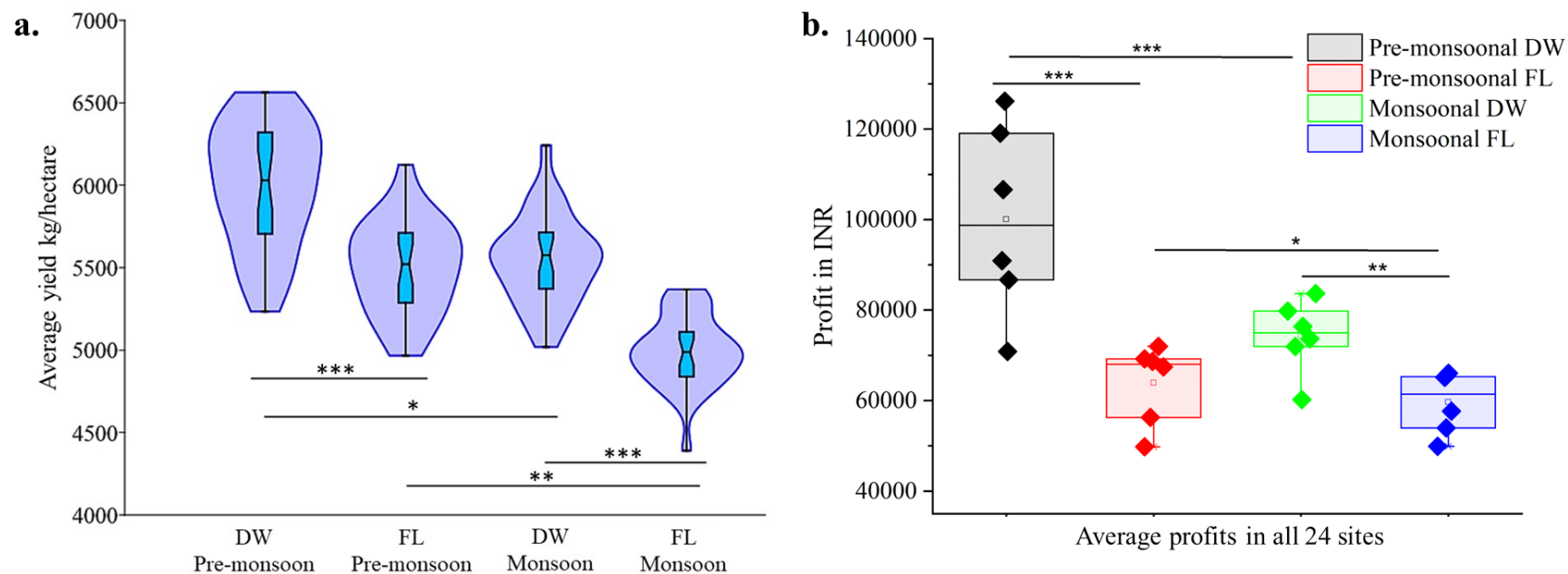


Fig. 2. Average rice yield and relative profit generated from all 24 sites under the compared practices. The average rice yield (kg/hectare) in all 24 sites under six Gangetic divisions is given in the form of a violin plot **(a)**. Based on these productivity records, monetary profit has been calculated for all 24 sites **(b)** indicating a much higher revenue generation using this AWD practice.



Fig. 3. Microbial Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolic pathways expressed differentially under dry-wet cycle and flooded cultivation. The KEGG database has been used to compare the obtained data from our high throughput sequencing to generate these heatmaps. To avoid excessive input in one file, the KEGG metabolic pathways are expressed in two separate images (**a, b**).

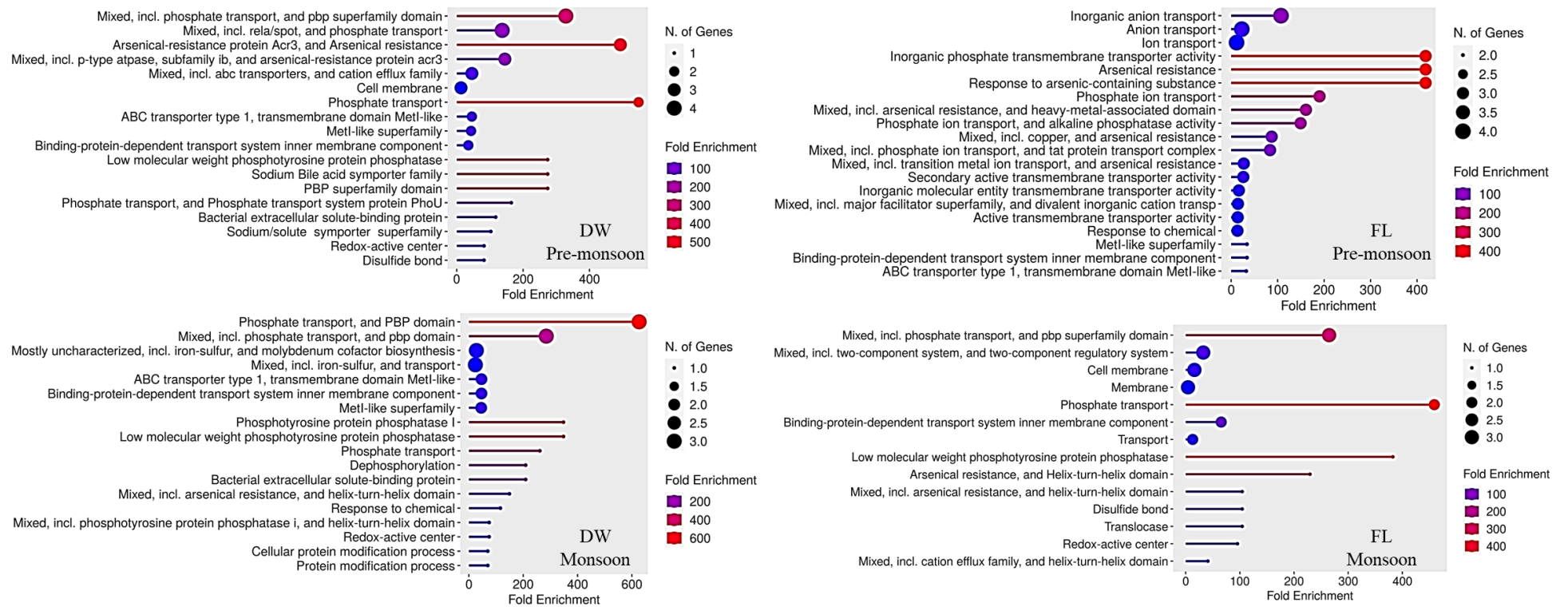


Fig. 5. Microbial arsenic-responsive and related gene activity with fold-change analysis. Arsenic-responsive and related gene prevalence, activity and fold-change occurrence in pre-monsoonal dry-wet and flooded along with monsoonal dry-wet and flooded microbes are presented in this figure.

Sustainable water management in rice cultivation reduces arsenic contamination, increases productivity, microbial molecular response, and profitability

Supplementary contents-

1. Additional methodologies.
2. Supplementary Figure S1. Study areas with sampling points of Upper middle to lower Gangetic delta of Indian subcontinent.
3. Supplementary Figure S2. Parametric-dependent pre-monsoonal and monsoonal distribution of soil As (a-d), Si (e-h).
4. Supplementary Figure S3. Stacked area plot for 24 sites with concentration range distribution of soil As and Si with altered irrigation. Part (a) indicates the results from pre-monsoonal sampling and (b) represents monsoonal sampling.
5. Supplementary Figure S4. Modelled soil As-Si complexation (a1-a2), modelled arsenic species (b-arsenate, b1-arsenite) and pourbaix diagram (c) of probable redox status within pH range 6.2 to 8.5.
6. Supplementary Figure S5. Database-made XRD analysis (a) and experimentally-proved As-Si association and availability depending on the organic matter (b) content in the soil.
7. Supplementary Figure S6. Rice plant vascular ultra-structure (Xy= xylem; Ph= phloem) under AWD and flooded conditions from 6 Gangetic divisions with 24 sampling sites (one figure from each site) indicating the role of higher Si and lower As accumulation effect on plant physiology.
8. Supplementary Figure S7. Rice yield productivity (a-f) and plant bioavailable As-Si concentrations (g-l) with spatial variation at 24 sites.
9. Supplementary Figure S8. Heatmap analysis of pre-monsoonal and monsoonal microbial class occurrence under two irrigation types.
10. Supplementary Figure S9. KEGG Metabolic pathway analysis with predominant biochemical modules as superimposed models of earlier reports. Our analysed pathways are highlighted with red lines in each diagram.
11. Supplementary Figure S10. Gene Ontology (GO) profiling of dominant microbial metabolisms with higher frequency (a) and lower frequency (b) activities.
12. Supplementary Figure S11. Arsenic responsive gene-dependent dominant microbial network analysis in pre-monsoonal dry-wet/flooded (a,b) and monsoonal dry-wet/flooded (c,d) phases respectively.
13. Supplementary Figure S12. Network relationship of As-responsive and related genes under pre-monsoonal dry-wet and flooded soils (a-b) and monsoonal dry-wet and flooded soils (c-d). The genes are named within the figure.
14. Supplementary Table S1. Standard reference material values as analyzed for quality assurance.
15. Supplementary Table S2. Soil texture analysis by ASTM sieve method of all 24 sites within a radius of 1km from the Ganga bed.
16. Supplementary Table S3. Alpha and Beta diversity indices of soil microbiome under different field setups and seasonal changes.

17. Supplementary Table S4. The volume of water (in L) which can be saved in AWD practice compared to the flooded.
18. Supplementary Table S5. Profit calculation based on the rice productivity per hectare (H) in dry-wet (DW) and flooded (FL) fields. Average data for India has been used collected from the Ministry of Agriculture database.
19. Supplementary Table S6. Profit calculations based on the average income per hectare from dry-wet and flooded fields.

Additional methodologies-

Experimental site design- Briefly, each field was 800m² in area and irrigated with groundwater during the pre-monsoonal season while rainwater was used in the monsoonal phase. Water stagnancy of 10cm was maintained for 2 days followed by a drying cycle with 60% soil moisture (time varied due to weather conditions) and repeated till the late inflorescence stage. During the monsoonal season, maintaining AWD was difficult but manageable, resulting in a marked difference.

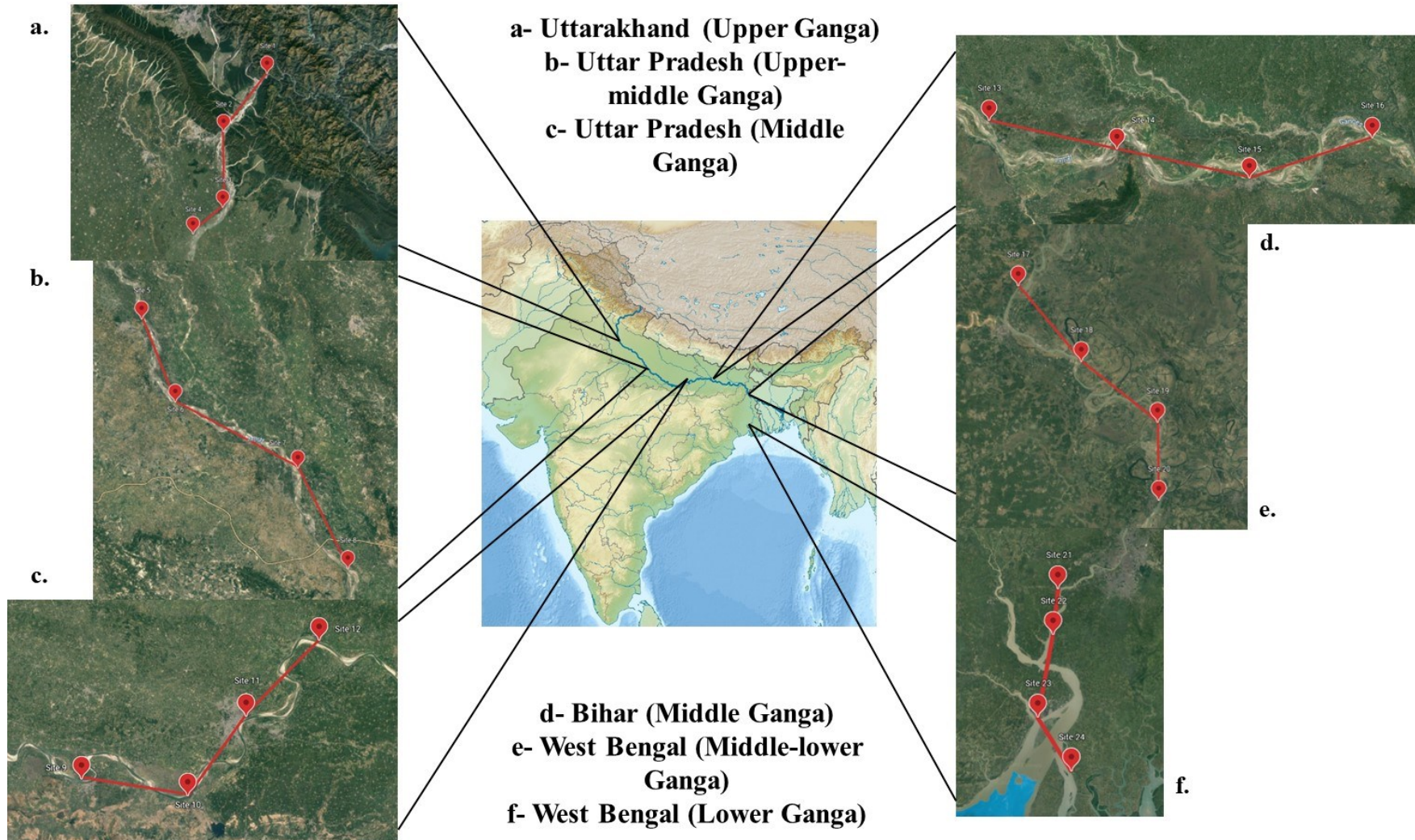
Soil-plant Si analysis- Briefly, soil extracts were mixed up with ammonium sulfomolybdate [(NH₄)₆Mo₇O₂₄·4H₂O]+H₂SO₄], tartaric acid (C₄H₆O₆) and ascorbic acid (C₆H₈O₆) at different concentrations as per the method and measured at 660nm using a spectrophotometer (Specord 210, AnalytikJena, Germany). Plant extracts were mixed up with hydrochloric acid (HCl), ammonium heptamolybdate [(NH₄)₆Mo₇O₂₄·4H₂O], and oxalic acid [(COOH)₂·2H₂O] and measured at 410nm. As a standard material for calibration, silicon tetrachloride (SiCl₄), NIST2711a and NIST1568c were used.

Secondary analyses- The pourbaix diagram of pH and ORP making a graph of the oxyhydroxide ions of As and Si in the soil-water interphase and during the transition phase (Majumdar et al., 2019). This online database allows generation of a single or multi-element molecular structure from obtained data and available crystal structures online. **Supplementary Figure S2** shows four such predicted As-Si molecular structures and a pourbaix diagram with predominant ions marked within the area of interest (red colored box). For further assessment of these oxyhydroxide ions of As and Si, experimentally obtained data were used to generate X-ray diffractograms on The Materials Project (**Supplementary Figure S3**). Generated peaks in these graphs were deduced further for the identification of the elemental groups present in the samples. The complementary part of Fig. S3 represents a bubble plot which was made using field-obtained OM, total soil As and Si data.

High throughput sequencing- High-throughput sequencing was performed using isolated DNA samples from collected soils using a Qiagen DNeasy Powersoil kit (Cat# 12888-50). Eluted DNAs in Nuclease-free water (Ambion Cat# AM9938) were further checked in Nanodrop 2000 for quality assurance. A Ligation sequencing kit (Oxford Nanopore Technologies, SQK-LSK109) and PCR Barcoding kit (Oxford Nanopore

Technologies, EXP-PCR096) were used for the library preparation for which purified amplicon DNAs were end-repaired by NEBnext Ultra II End Repair kit (New England Biolabs, MA, USA) followed by a cleaning step using AmPure beads (Beckmann Coulter, USA). Barcoded DNA samples were further pooled and quantified using NEBnext Ultra II End repair/dA-Tailing Module (New England Biolabs, MA, USA). Another set of cleaning and adapter ligation was done using AmPure beads and NEB blunt/TA Ligase (New England Biolabs, MA, USA). Finally, the sequencing was conducted on GridION X5 (Oxford Nanopore Technologies, Oxford, UK) using SpotON flow cell R9.4 (FLO-MIN106). Nanopore raw reads ('fast5' format) were basecalled ('fastq' format) and demultiplexed using Guppy v2.3.4. These processed data are used further for the microbial expressions of metabolic and gene ontological status.

Supplementary Figure S1. Study areas with sampling points of Upper middle to lower Gangetic delta of Indian subcontinent.

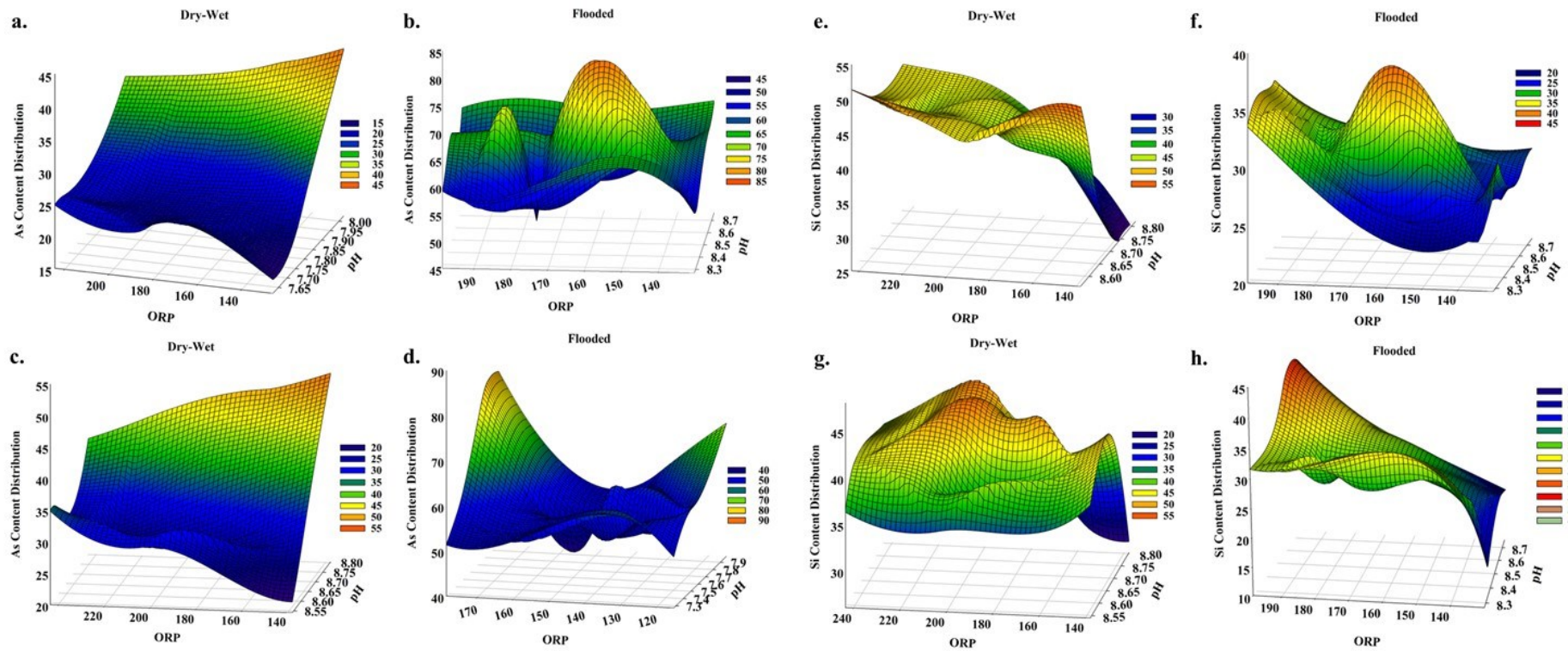


The coordinates of these 24 sampling sites are given below, which have been divided into six Gangetic divisions- Upper Ganga, Upper-middle Ganga, Middle Ganga 1, Middle Ganga 2, Lower-middle Ganga and Lower Ganga. Sampling sites are in Uttarakhand, Uttar Pradesh, Bihar and West Bengal.

Upper Ganga	Upper-middle Ganga	Middle Ganga 1	Middle Ganga 2	Middle-lower Ganga
30D08'24"N 78D19'53"E	27D24'29"N 79D36'54"E	25D10'00"N 82D33'12"E	25D28'29"N 85D57'22"E	23D42'10"N 88D08'44"E
29D57'24"N 78D09'17"E	27D14'52"N 79D41'59"E	25D07'26"N 82D50'36"E	25D22'12"N 86D27'58"E	23D36'02"N 88D15'14"E
29D42'30"N 78D09'29"E	27D06'52"N 79D58'46"E	25D20'03"N 83D01'45"E	25D16'48"N 86D58'33"E	23D30'58"N 88D22'05"E
29D37'19"N 78D03'10"E	26D53'14"N 80D06'45"E	25D32'03"N 83D14'16"E	25D24'16"N 87D27'08"E	23D25'18"N 88D22'59"E
Lower Ganga				
22D28'17"N 88D06'43"E				
22D18'38"N 88D06'13"E				
22D01'15"N 88D03'32"E				
21D50'12"N 88D11'46"E				

Three random replicates have been sampled from each of these sites to maintain the sampling process's heterogeneity.

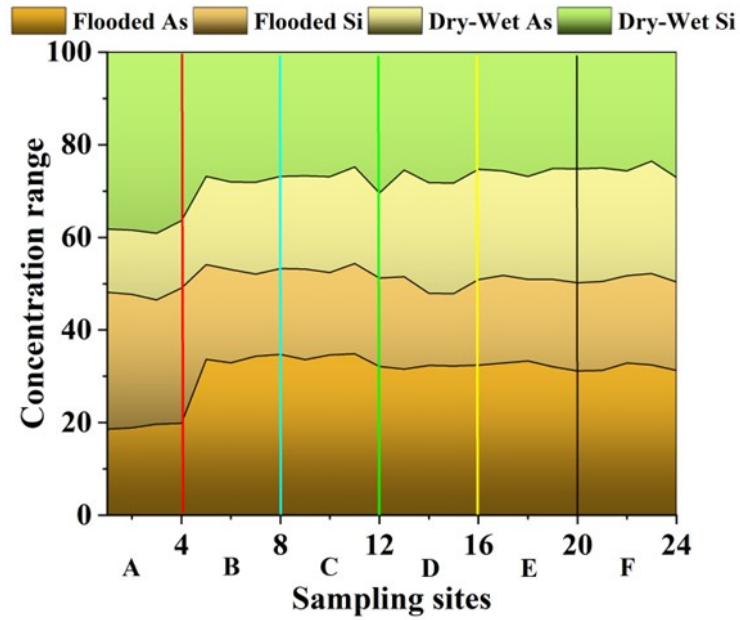
Supplementary Figure S2. Parametric-dependent pre-monsoonal and monsoonal distribution of soil As (a-d), Si (e-h).



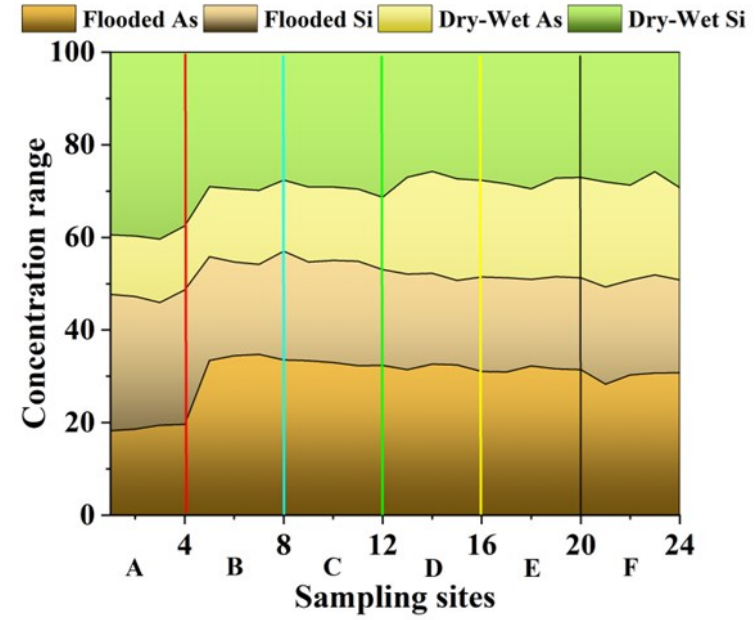
Figures 1a-b and 1c-d show pre-monsoonal and monsoonal soil As data in dry-wet and flooded conditions. Figures 1e-f and 1g-h show pre-monsoonal and monsoonal soil Si data in dry-wet and flooded conditions.

Supplementary Figure S3. Stacked area plot for 24 sites with concentration range distribution of soil As and Si with altered irrigation. Part (a) indicates the results from pre-monsoonal sampling and (b) represents monsoonal sampling.

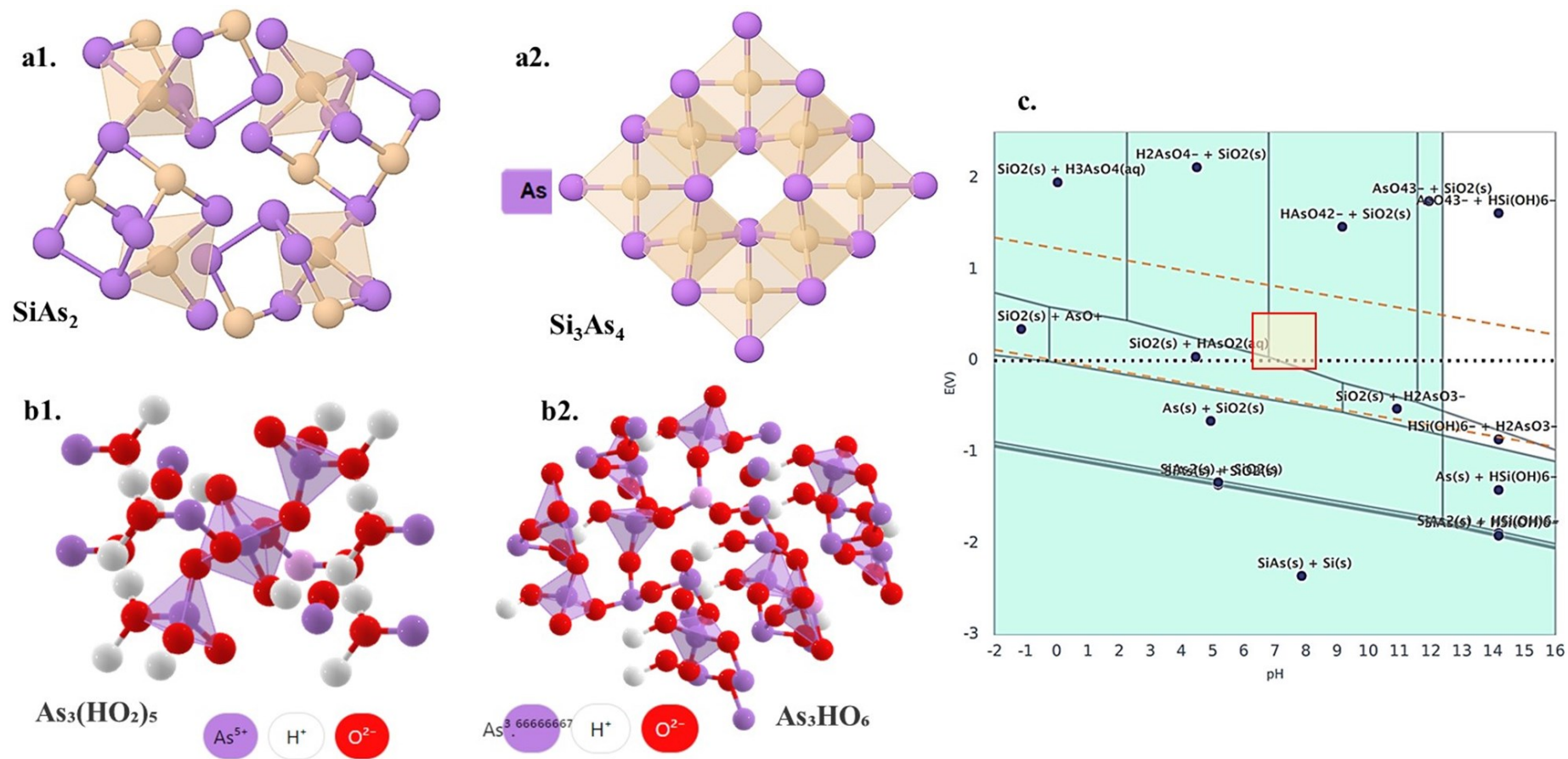
a.



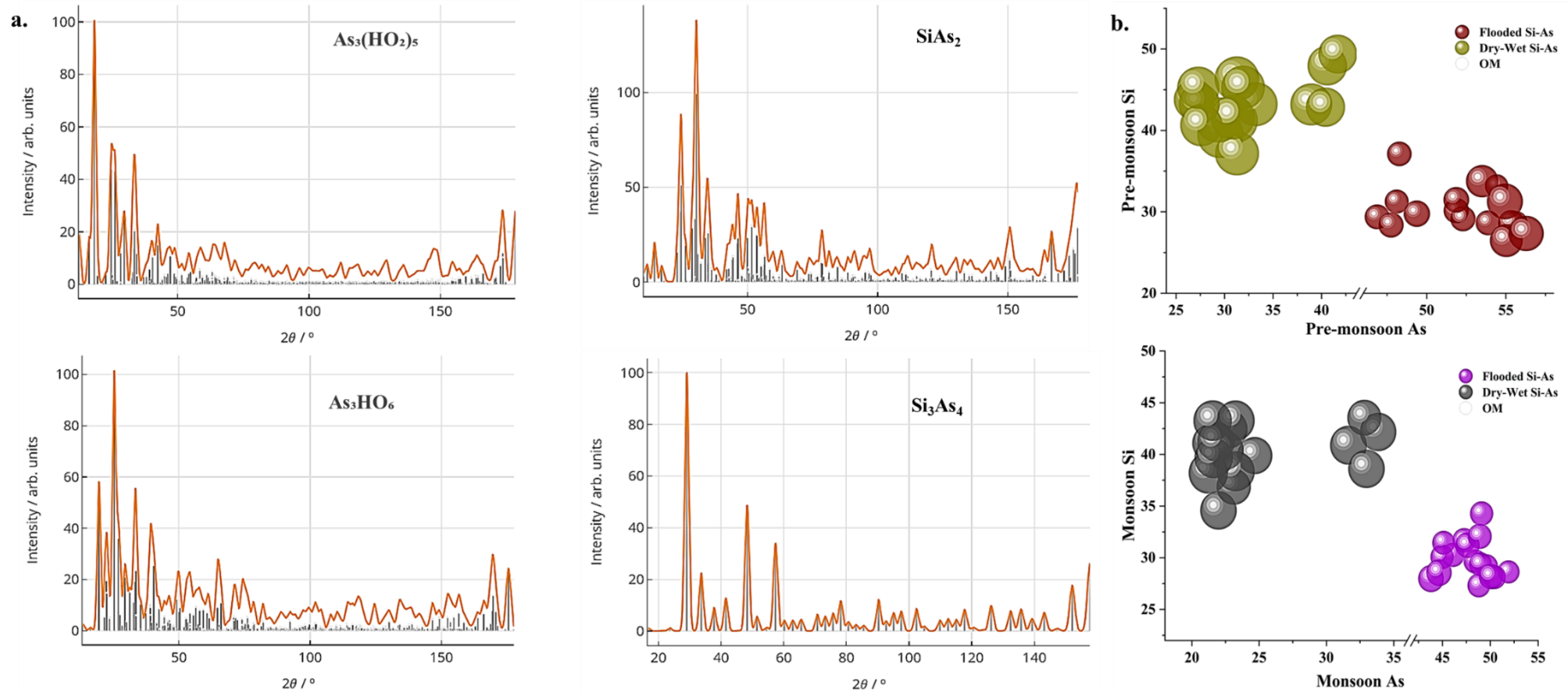
b.



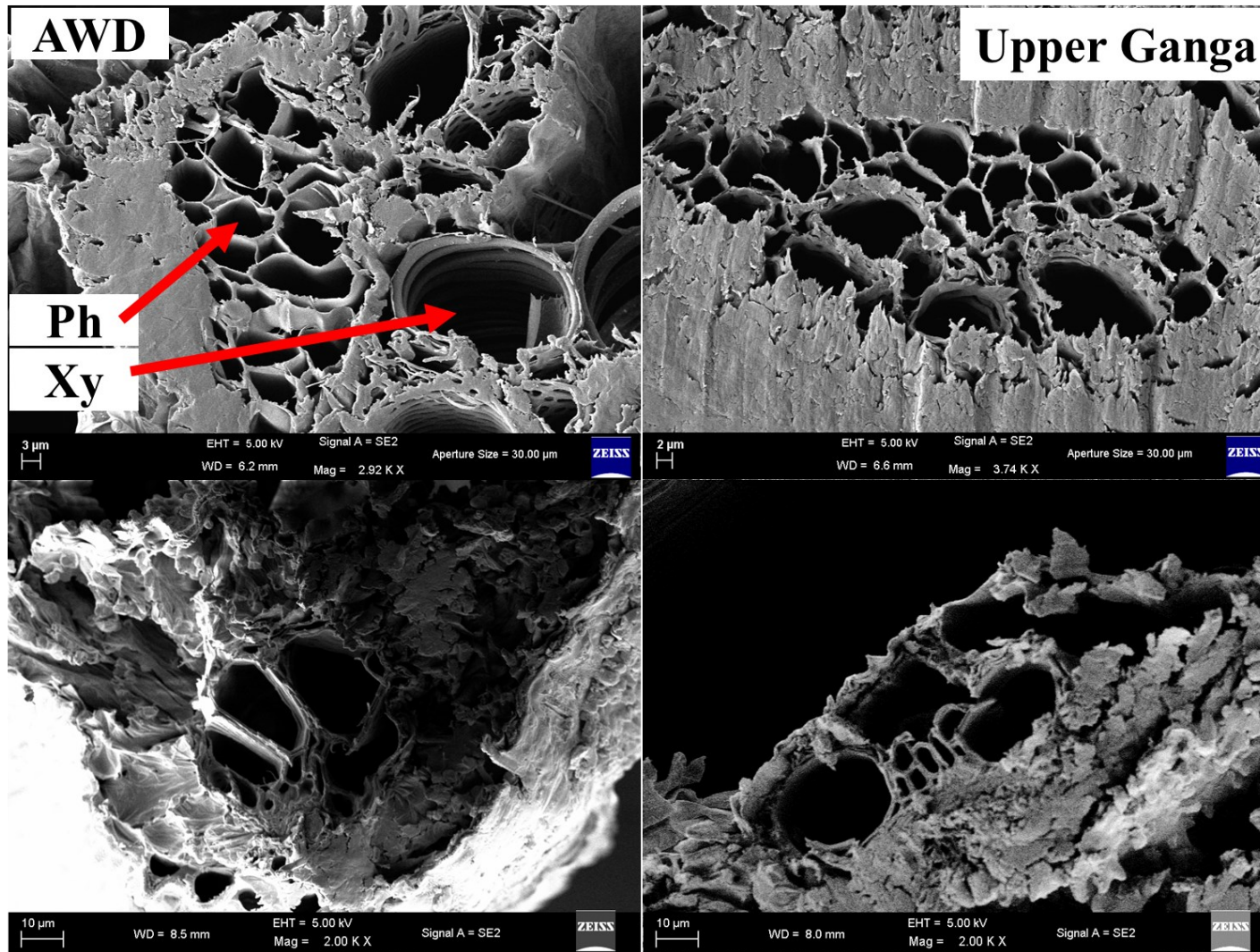
Supplementary Figure S4. Modelled soil As-Si complexation (a1-a2), modelled arsenic species (b-arsenate, b1-arsenite) and pourbaix diagram (c) of probable redox status within pH range 6.2 to 8.5.

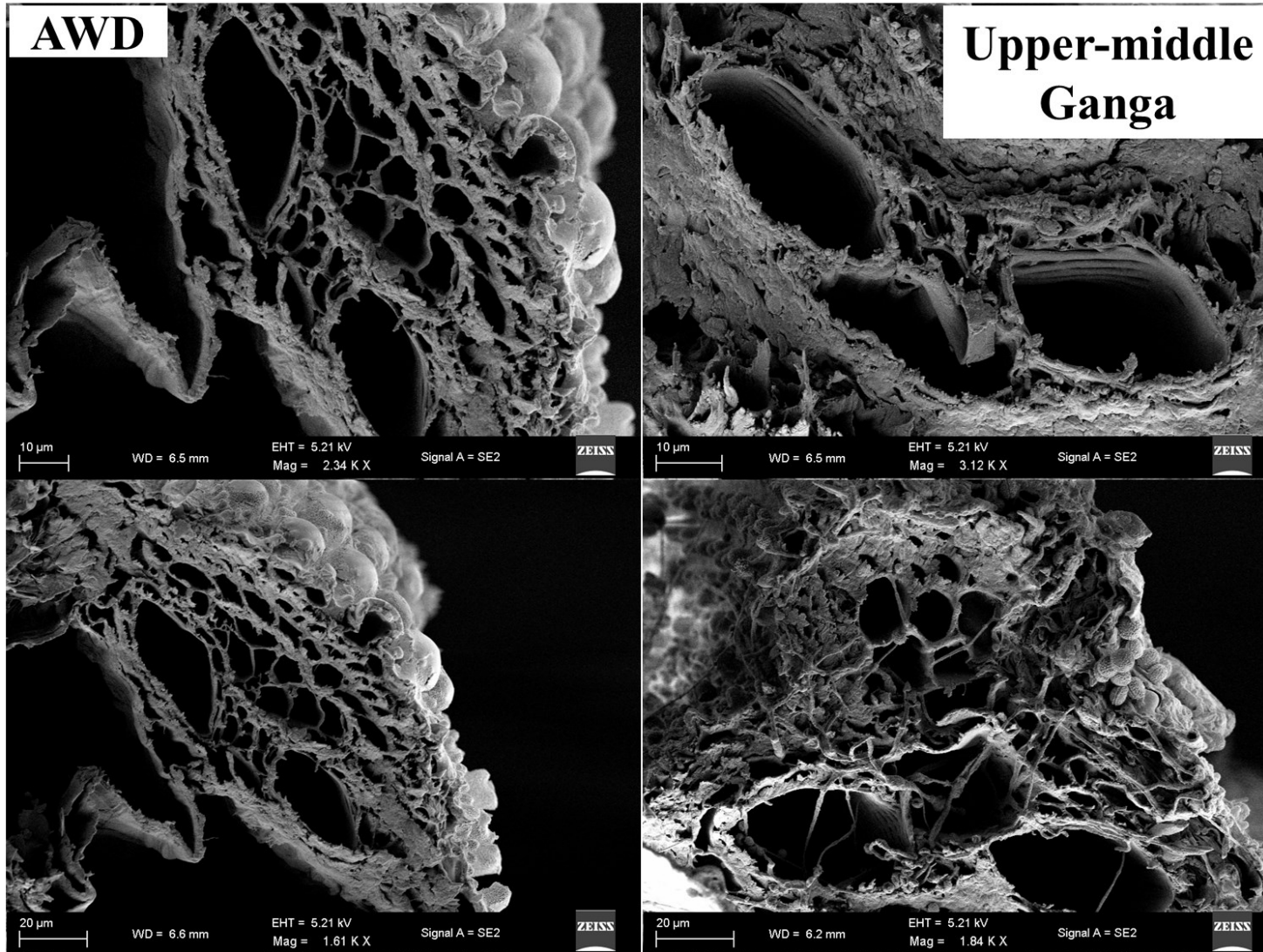


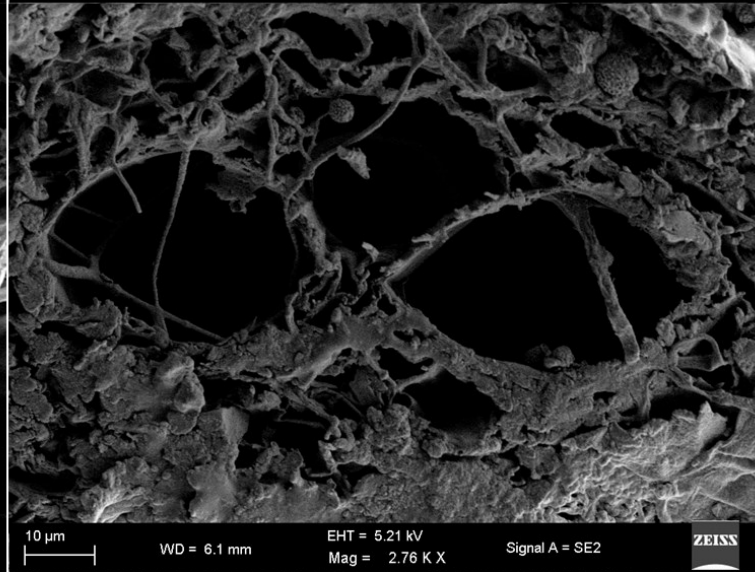
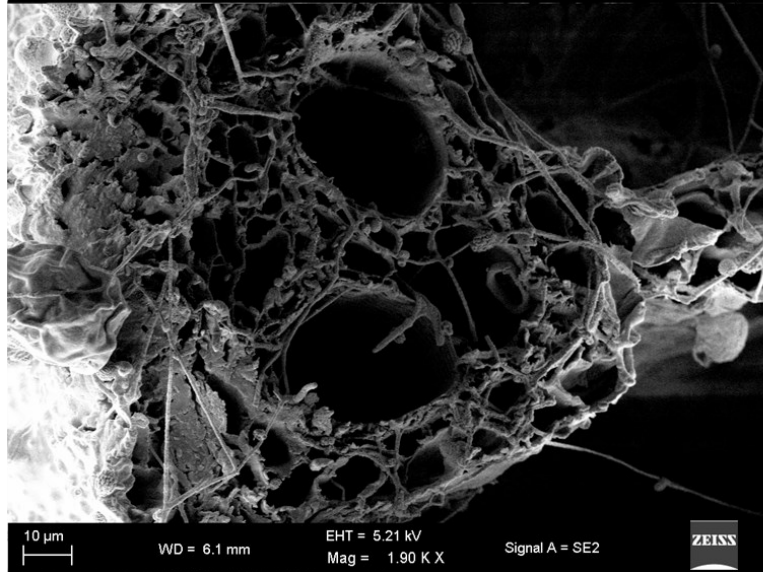
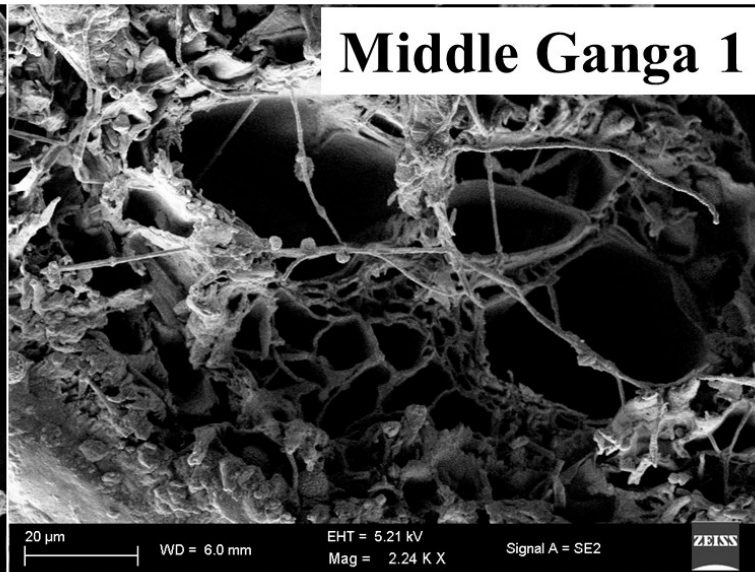
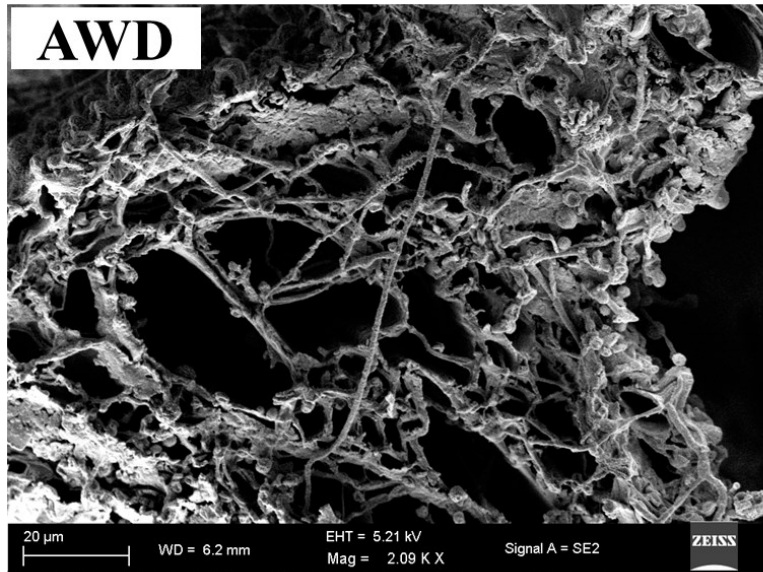
Supplementary Figure S5. Database-made XRD analysis (a) and experimentally-proved As-Si association and availability depending on the organic matter (b) content in the soil.

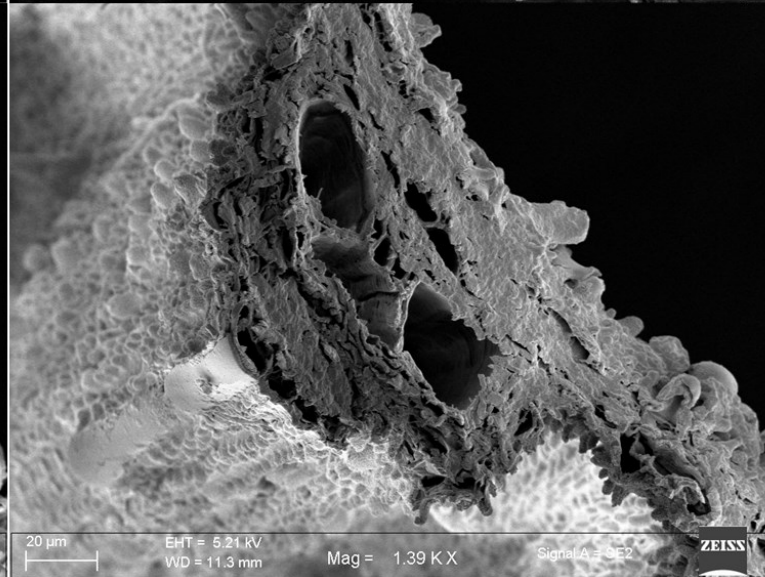
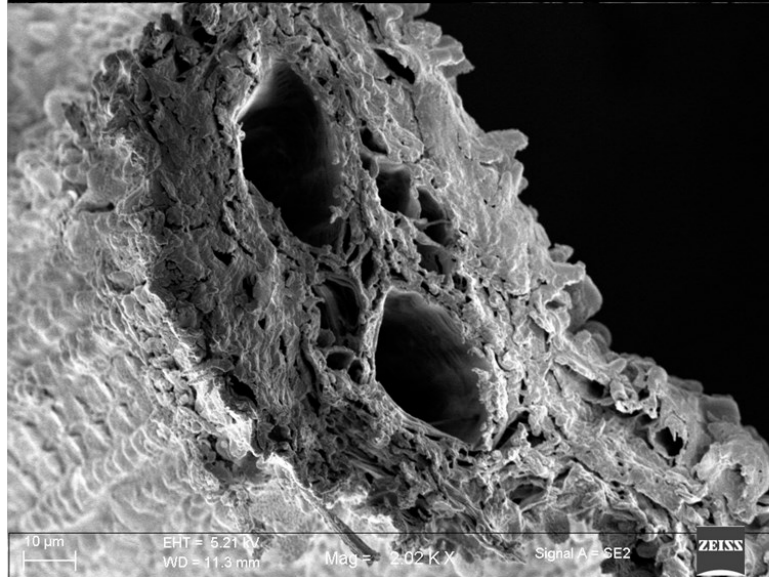
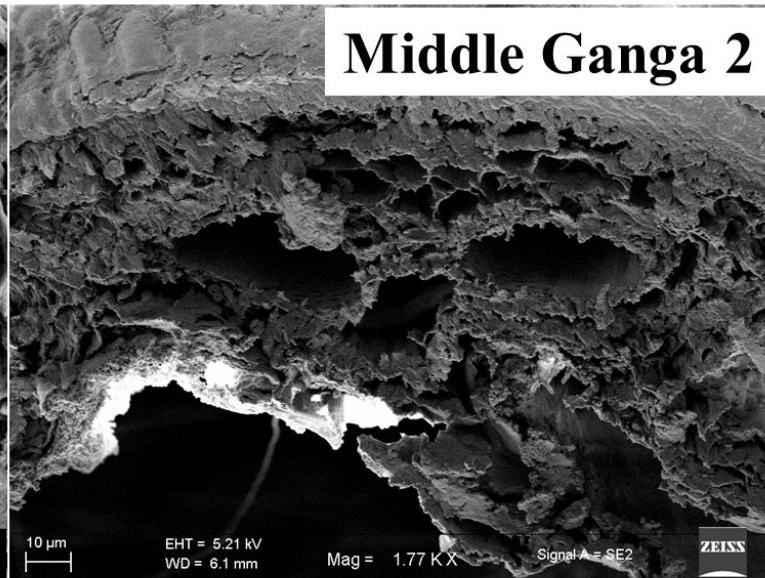
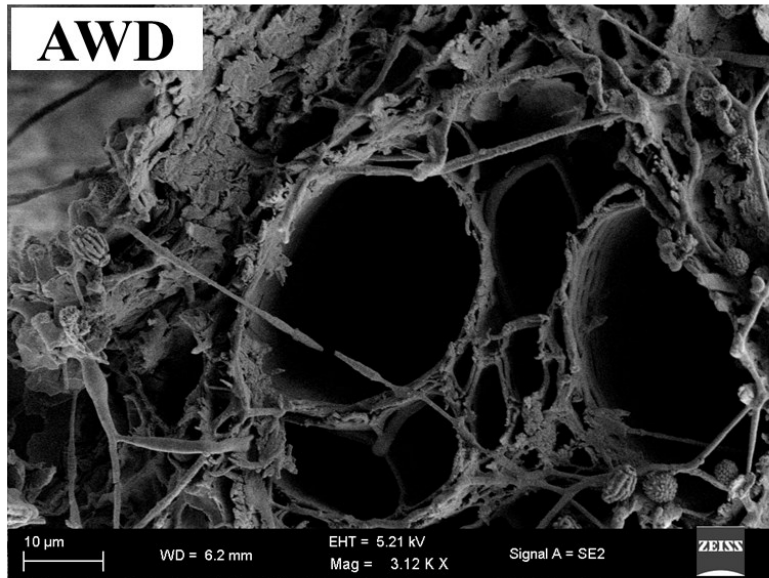


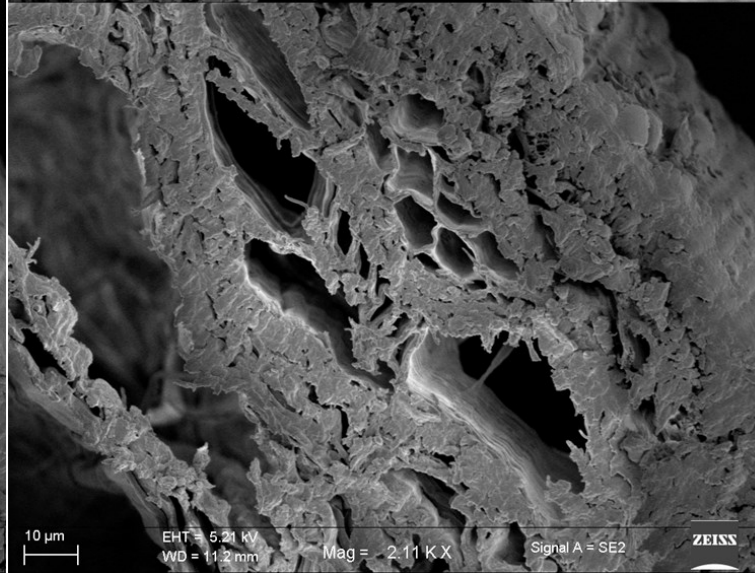
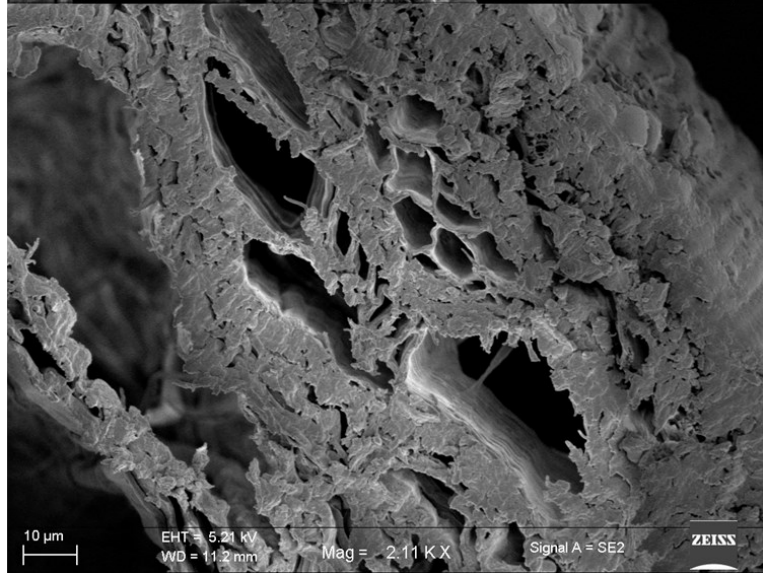
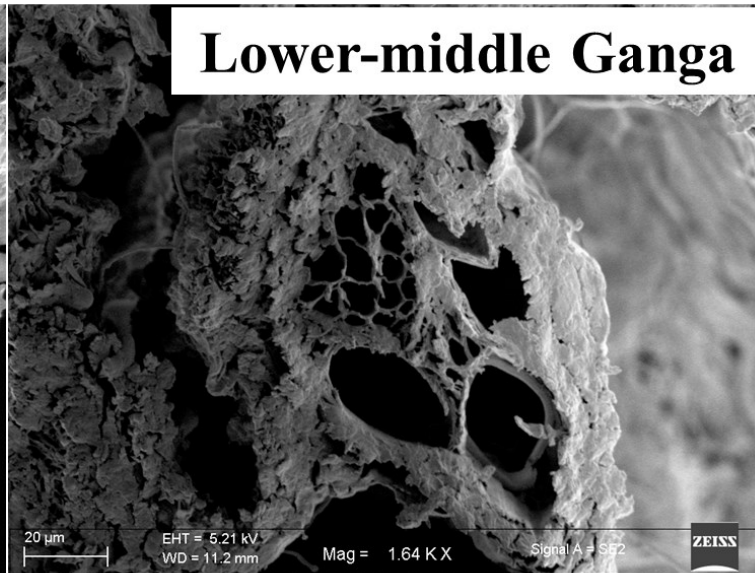
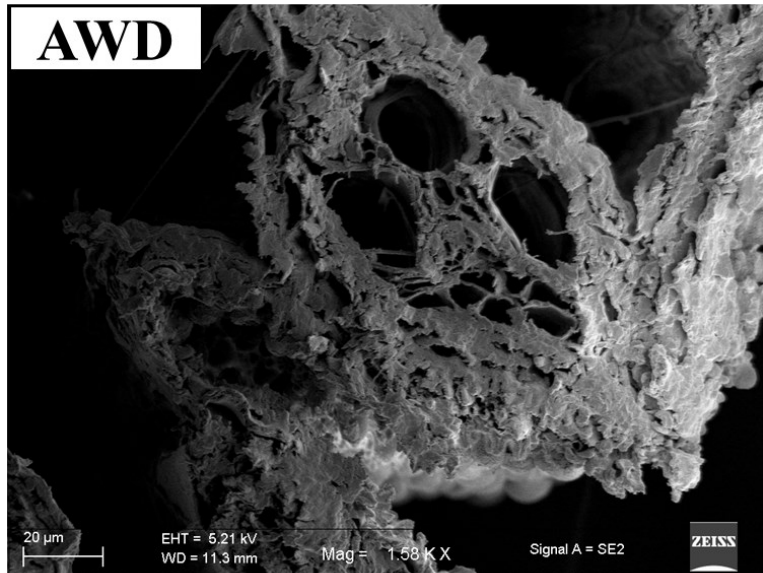
Supplementary Figure S6. Rice plant vascular ultra-structure (Xy= xylem; Ph= phloem) under AWD and flooded conditions from 6 Gangetic divisions with 24 sampling sites (one figure from each site) indicating the role of higher Si and lower As accumulation effect on plant physiology.

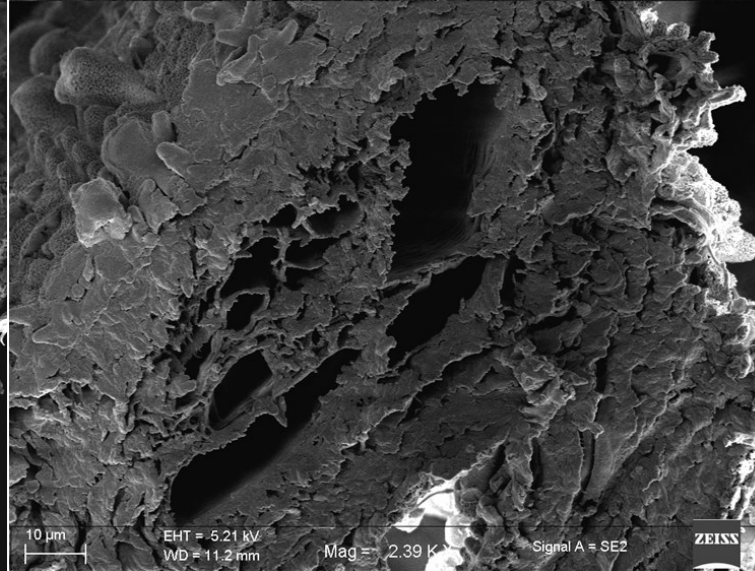
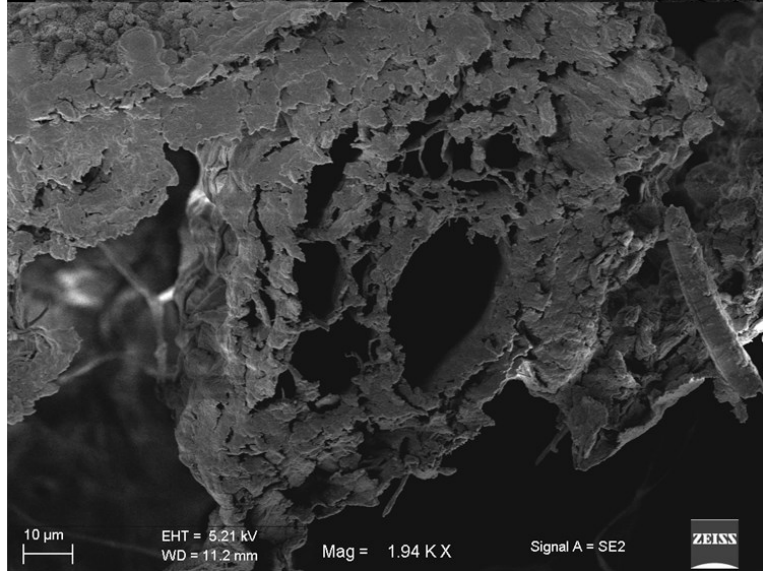
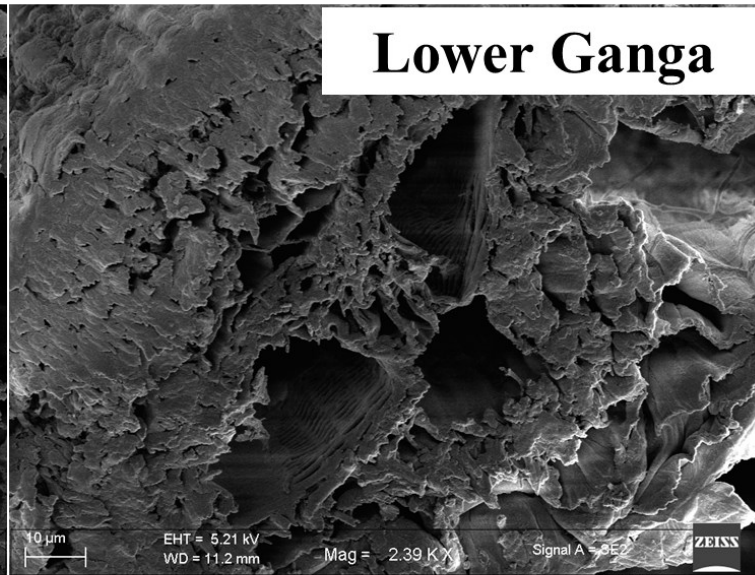
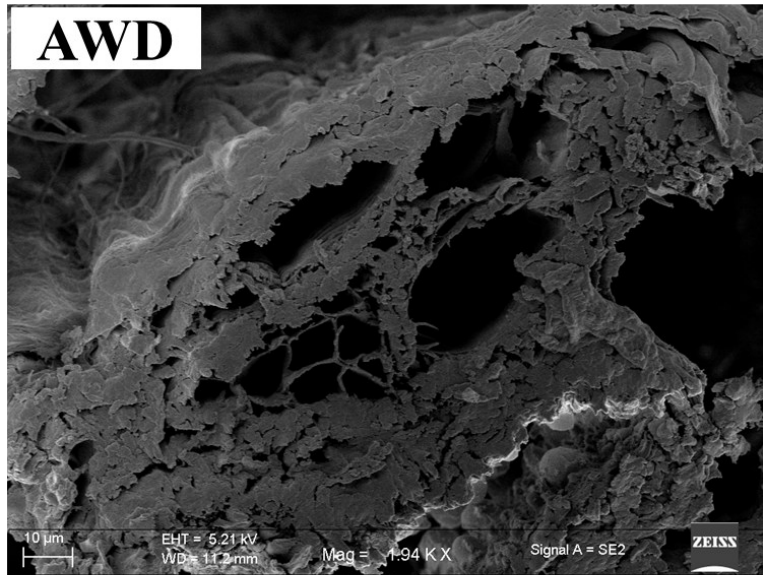


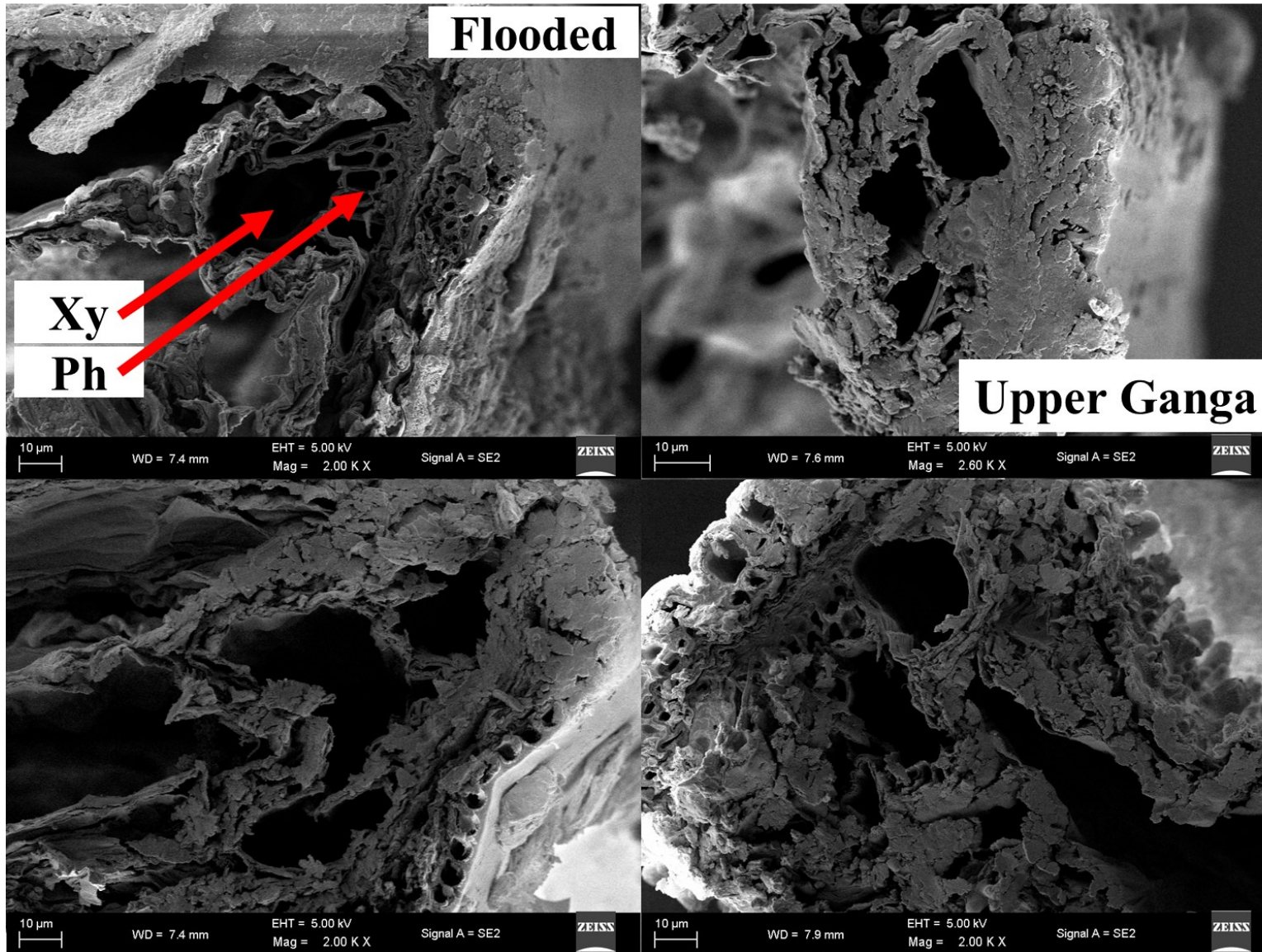






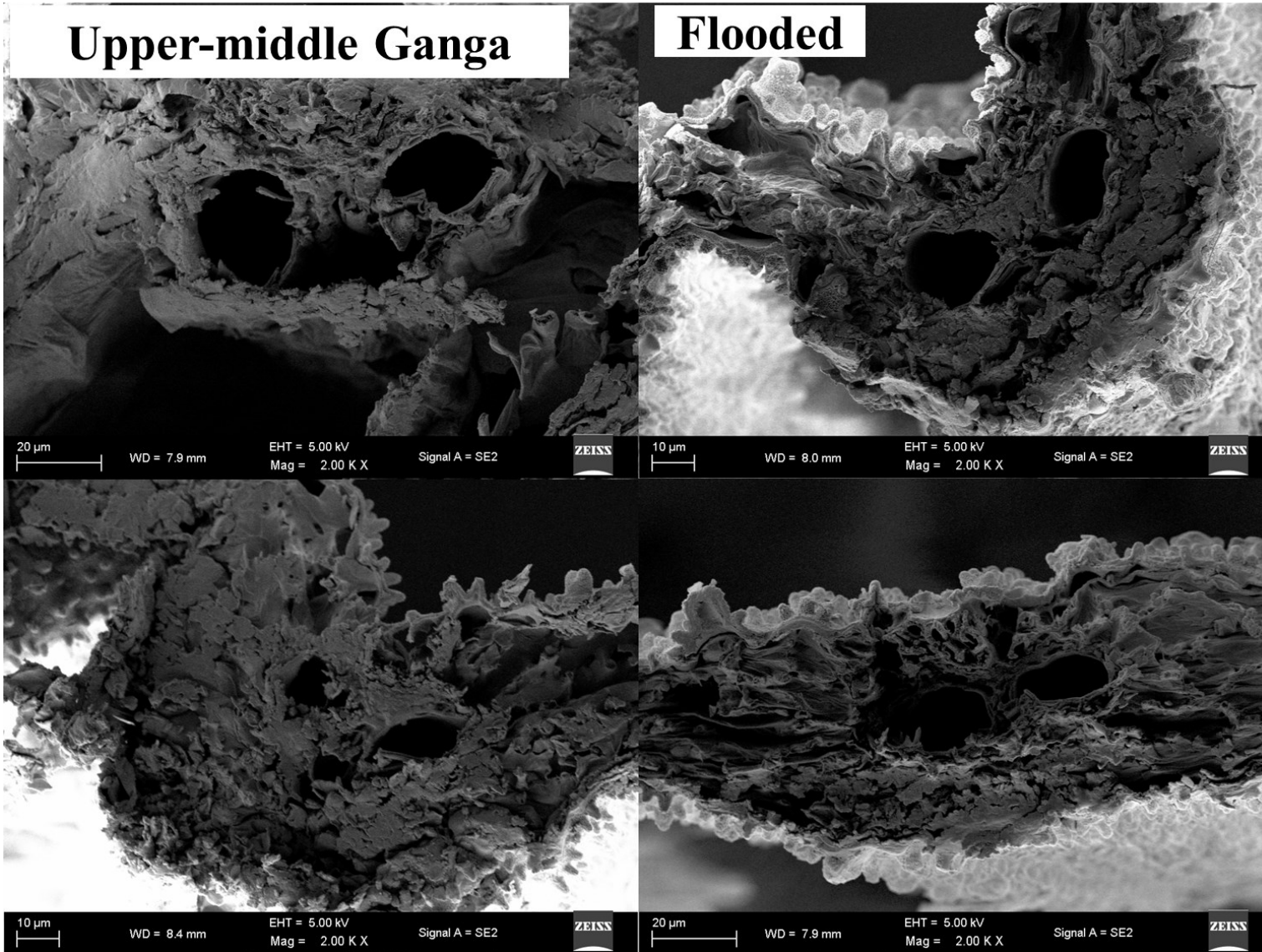


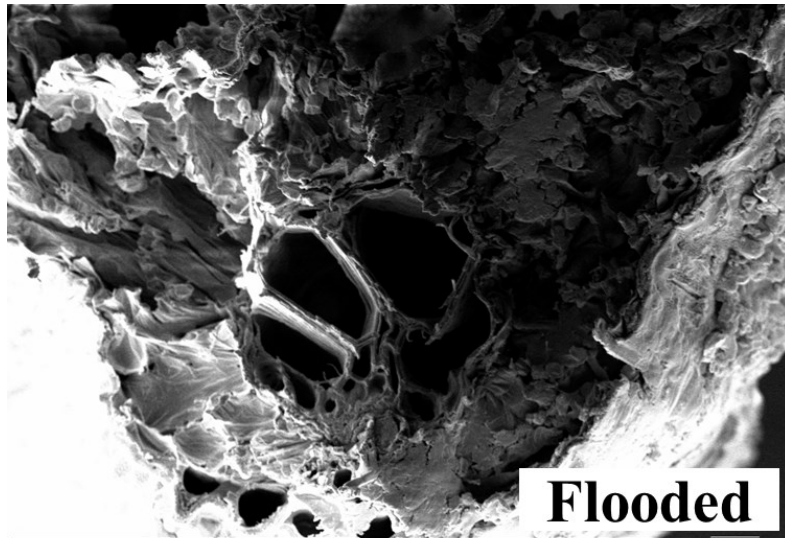




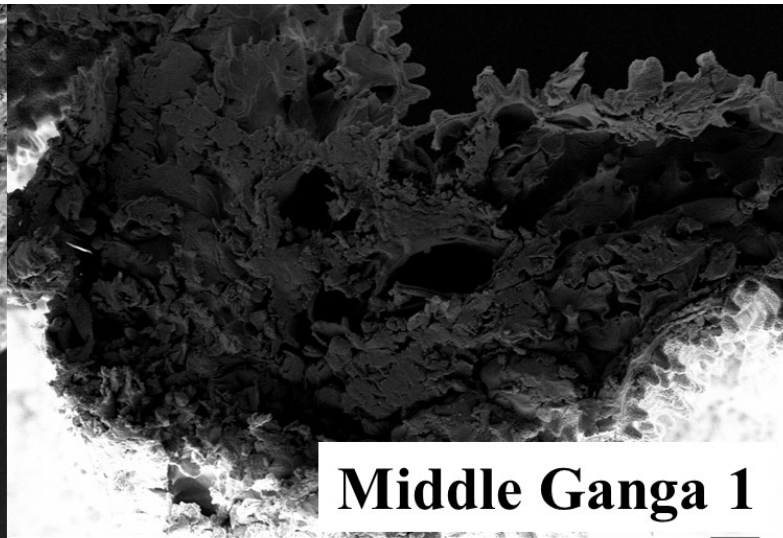
Upper-middle Ganga

Flooded

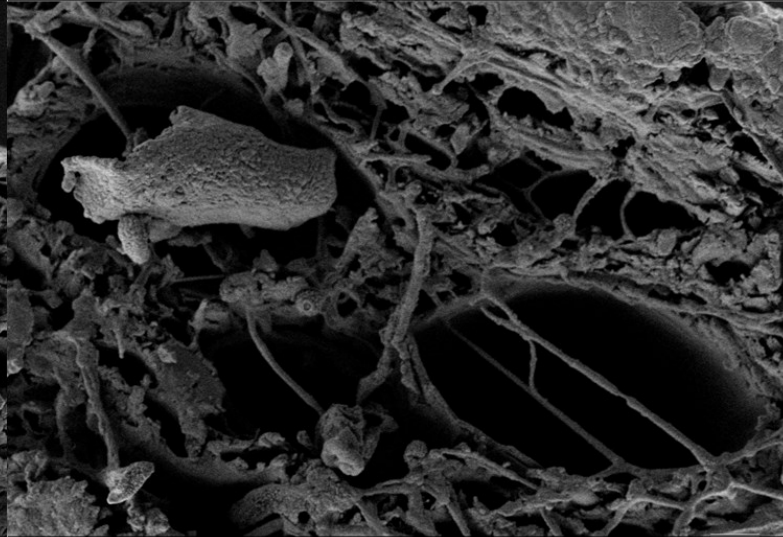
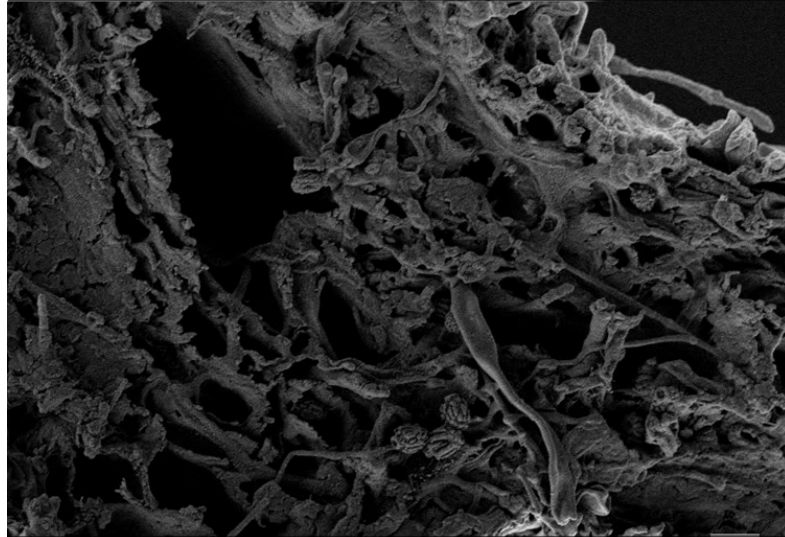


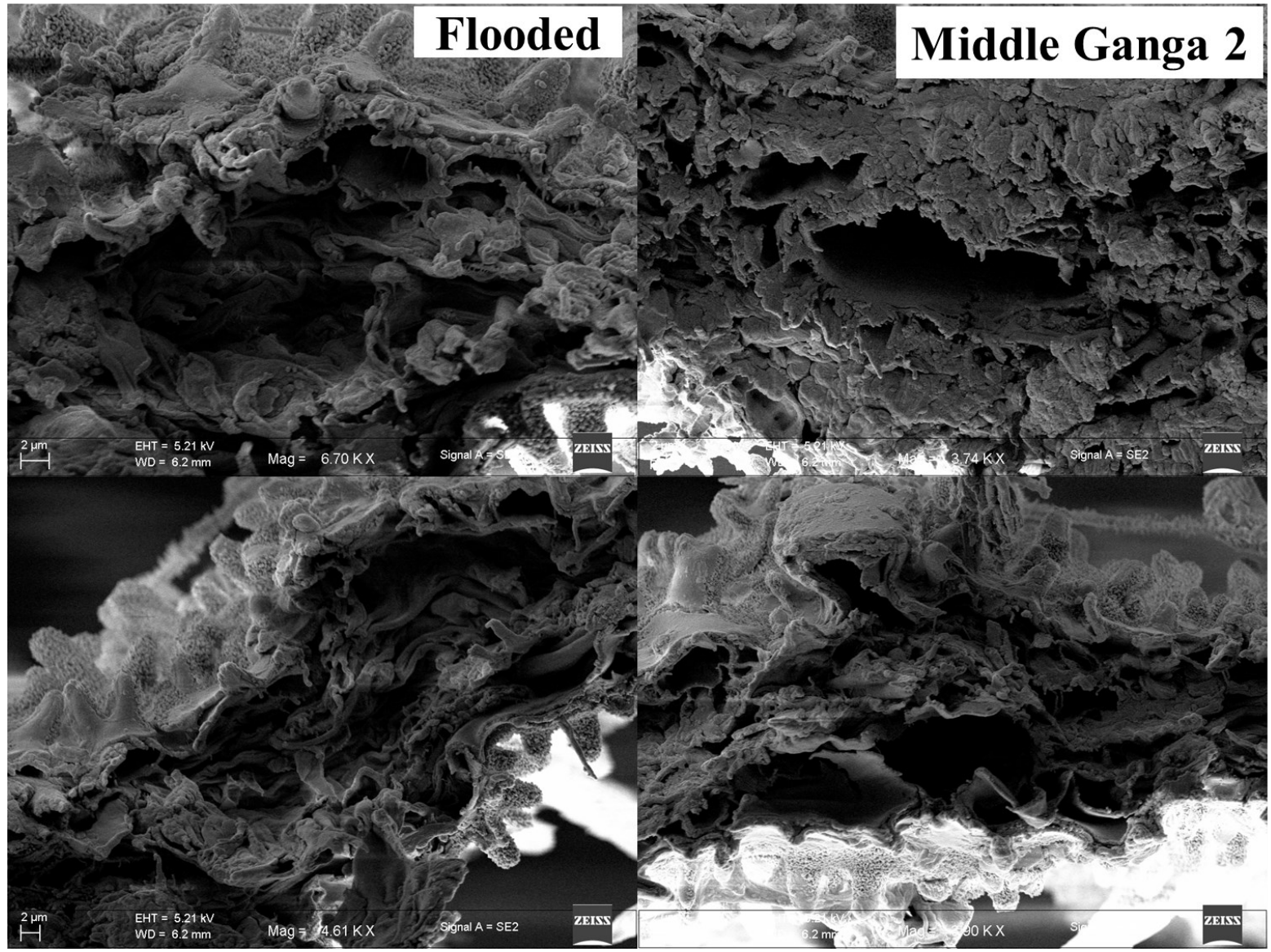


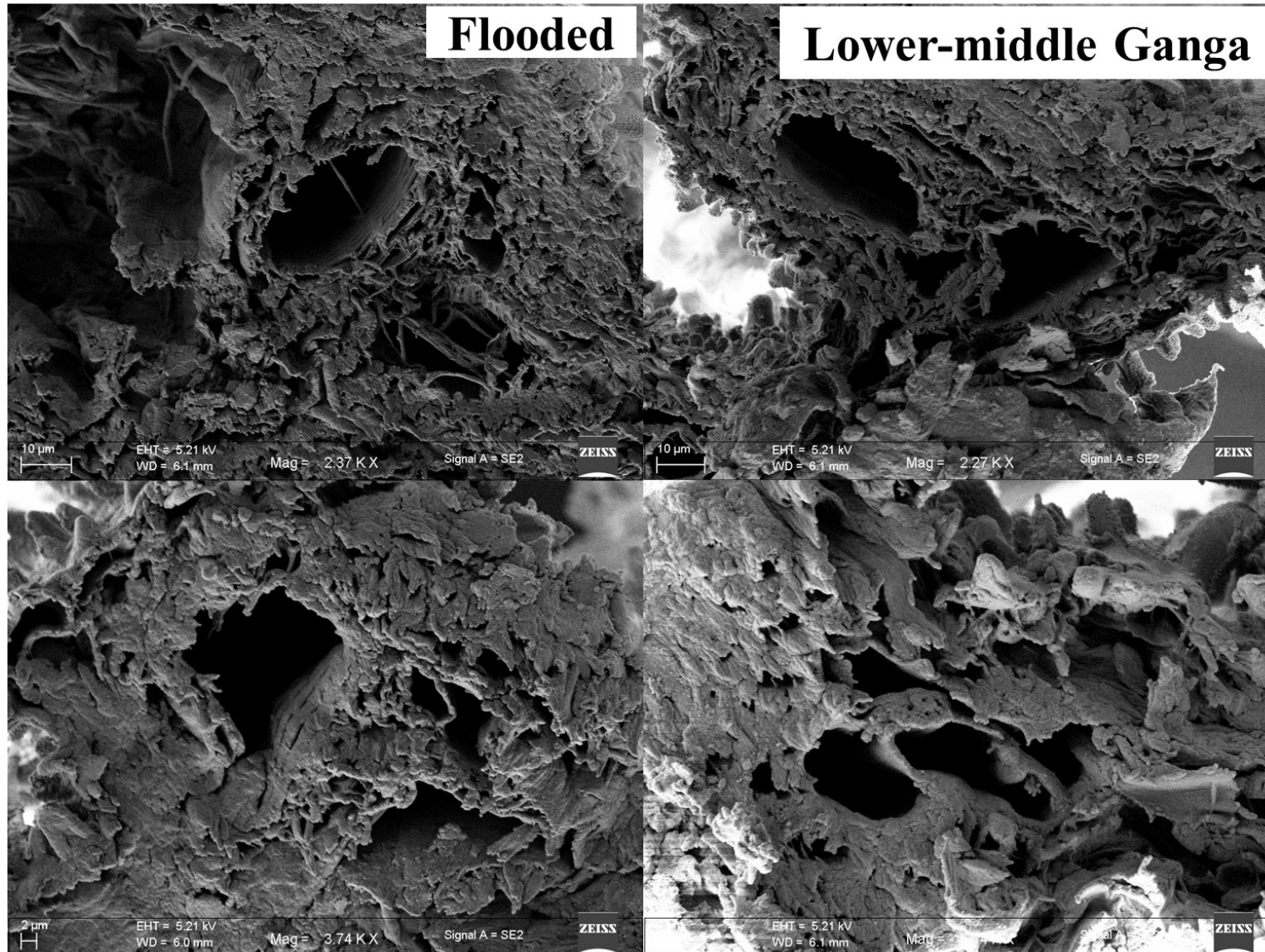
10 μ m | WD = 8.5 mm | EHT = 5.00 kV | Mag = 2.00 K X | Signal A = SE2 | ZEISS

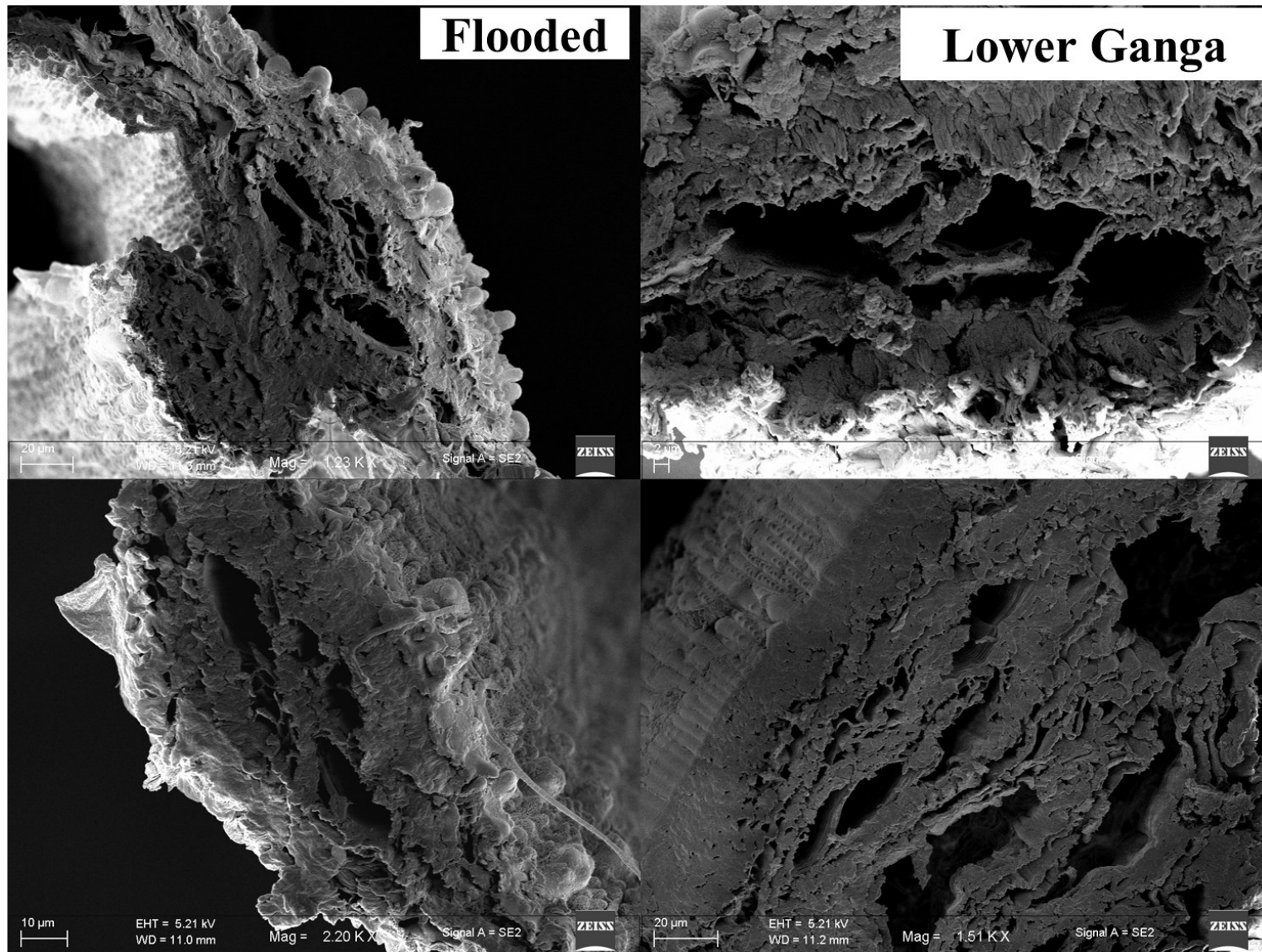


10 μ m | WD = 8.5 mm | EHT = 5.00 kV | Mag = 2.00 K X | Signal A = SE2 | ZEISS

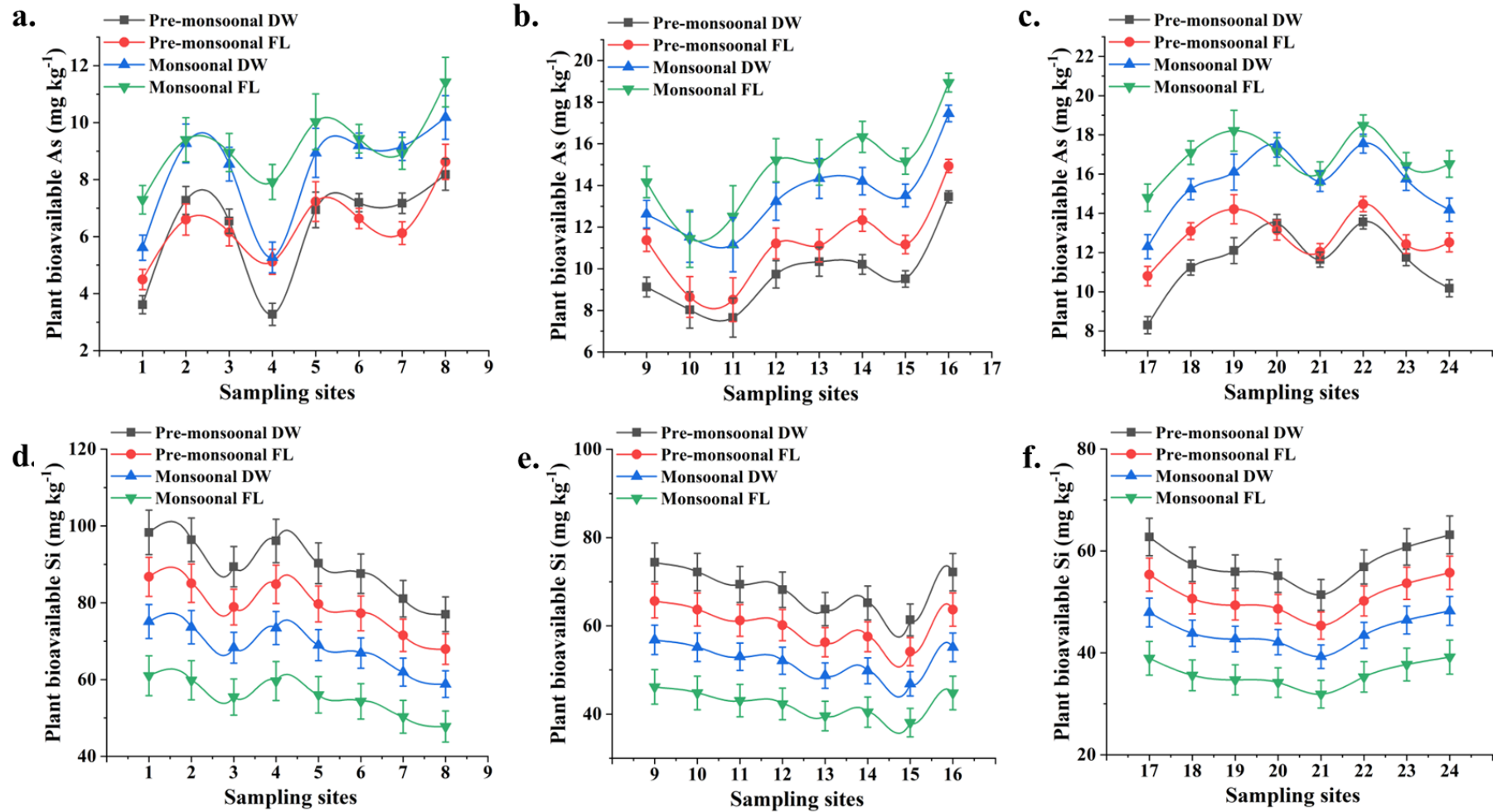








Supplementary Figure S7. Rice yield productivity (a-f) and plant bioavailable As-Si concentrations (g-l) with spatial variation at 24 sites.



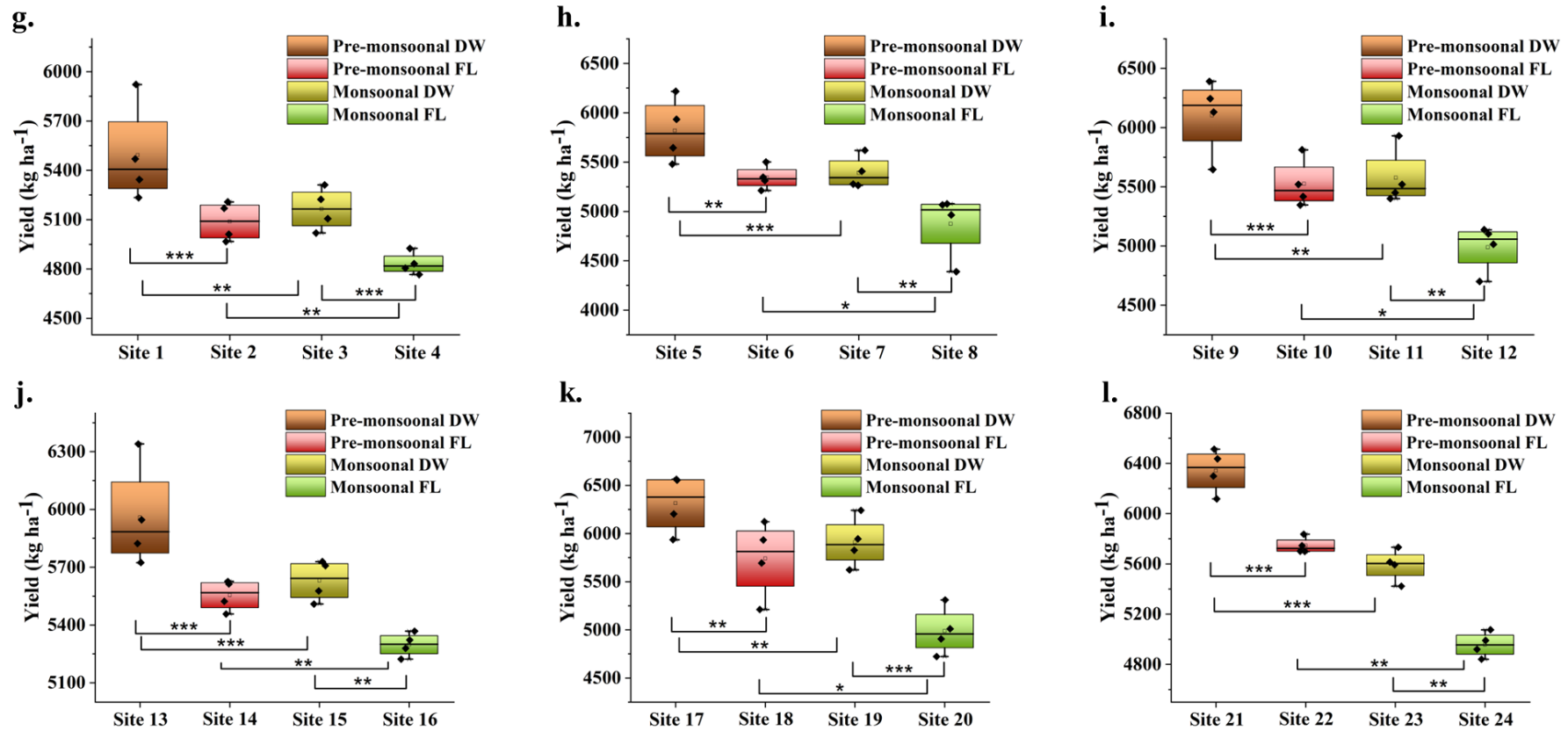
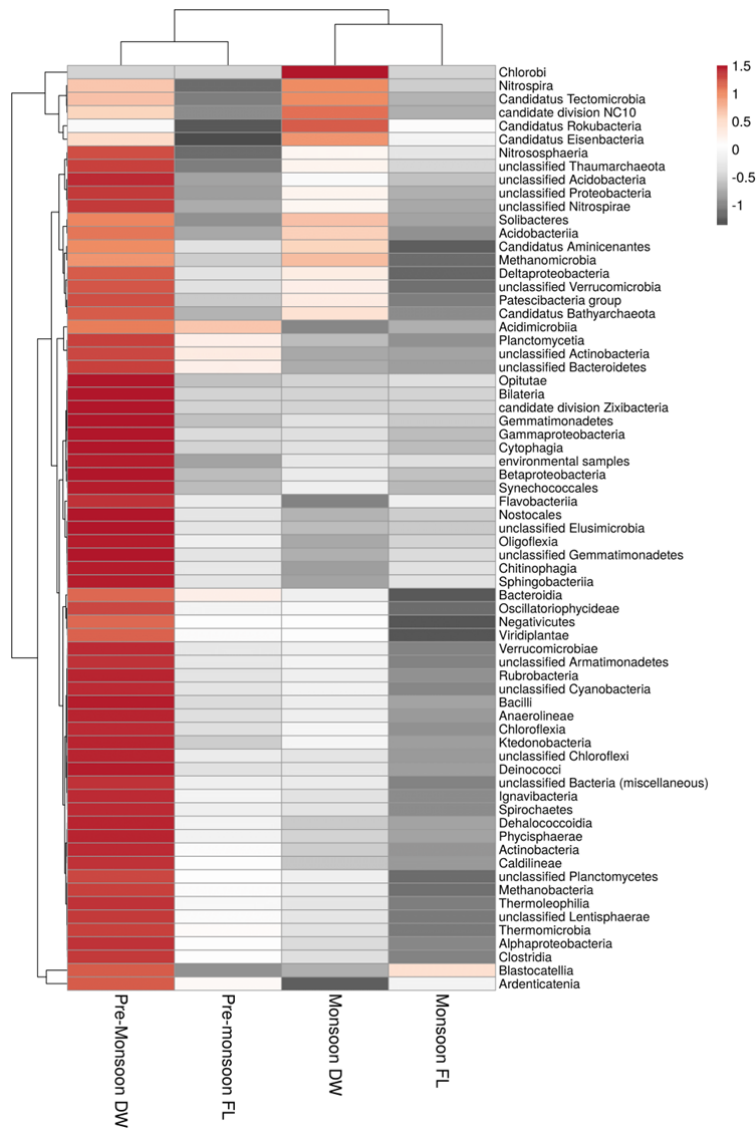
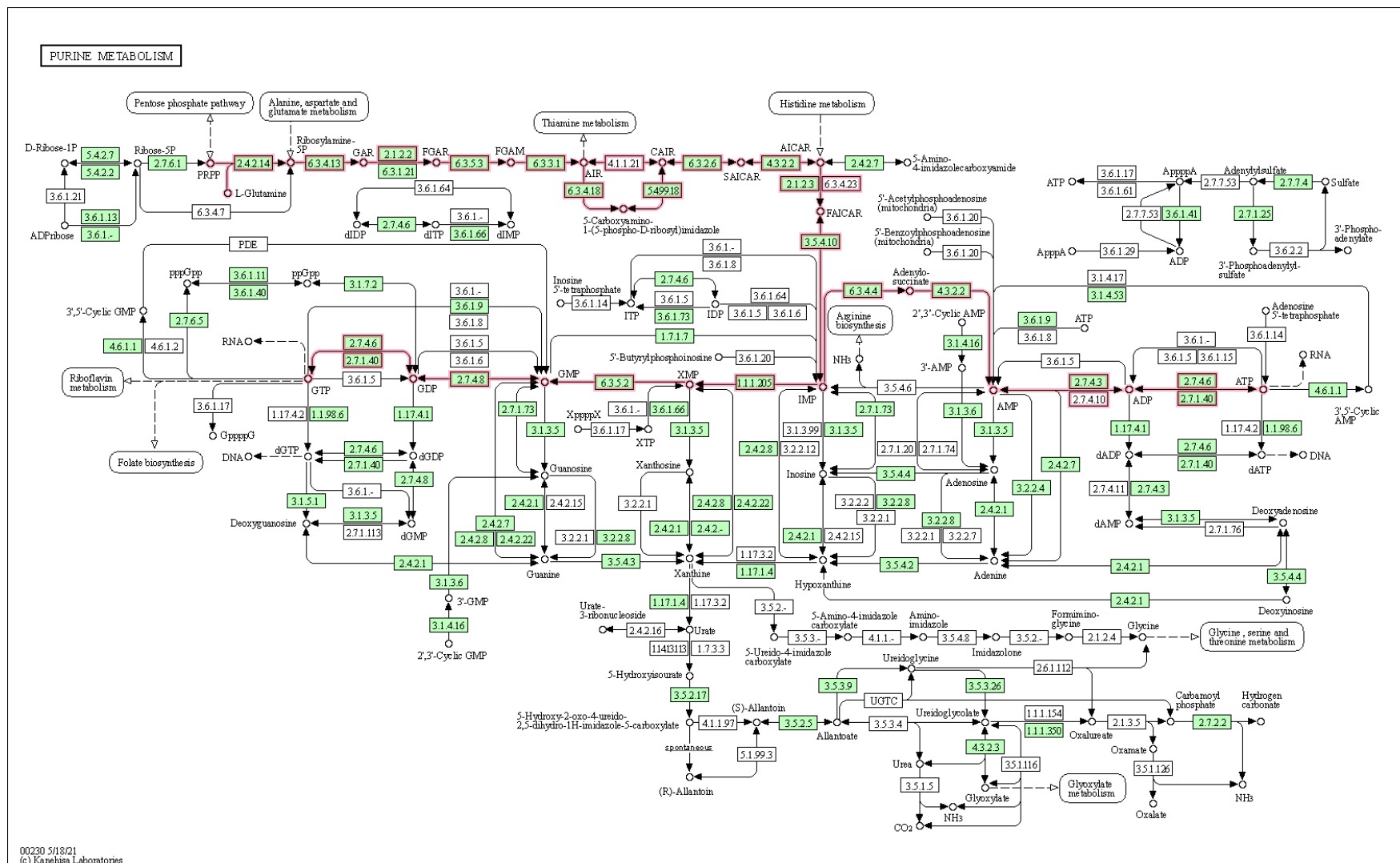


Figure 1a-f shows the As and Si bioavailable concentrations in soil under both the irrigation regimes and seasonal change. Each site represents the mean yield data (1g-l) of triplicate sampling points where significant variance has been presented with ‘***’ to be highly significant, ‘**’ moderate significance and ‘*’ low significance. Graphs are representative of triplicate data with standard deviation.

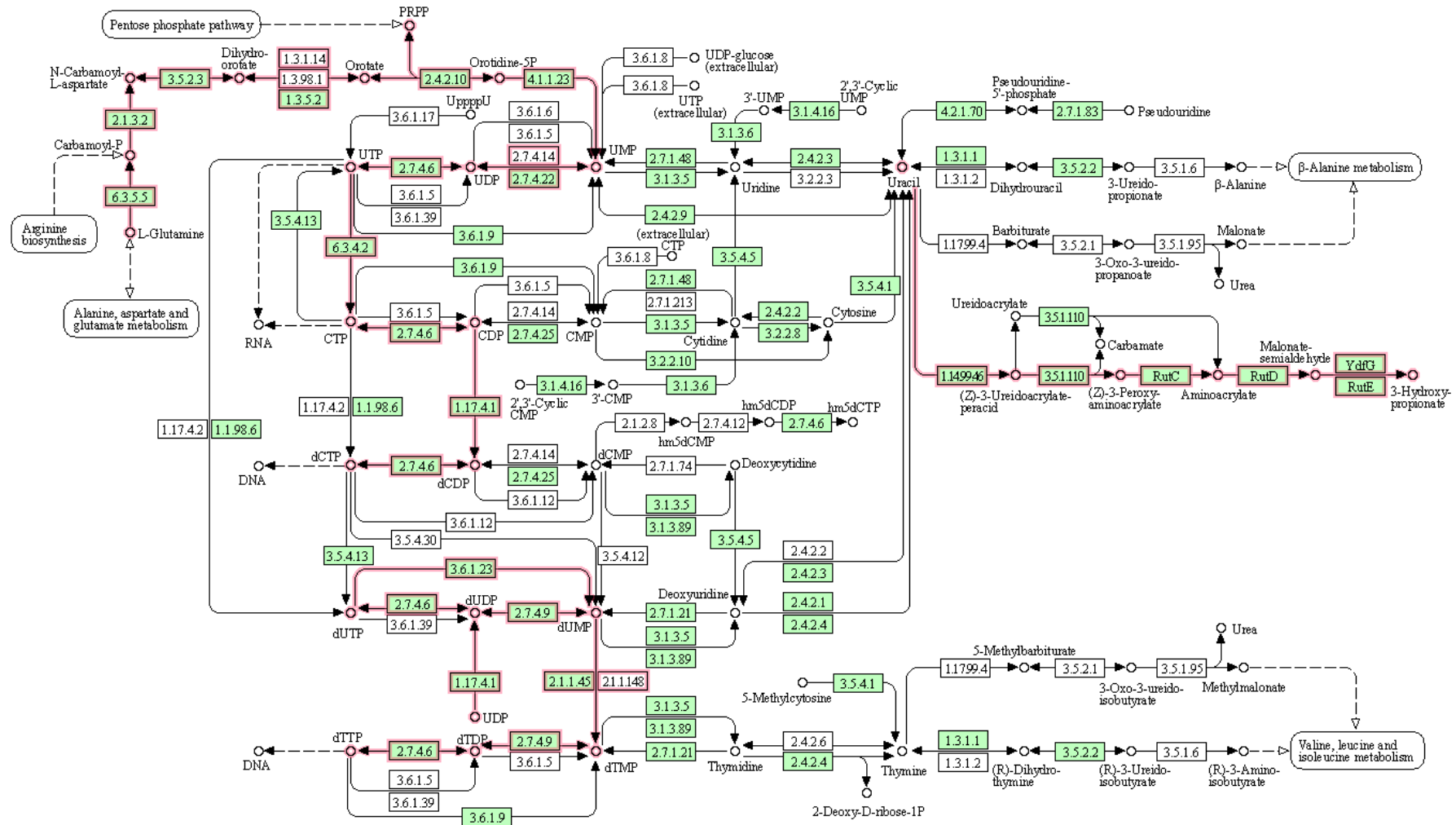
Supplementary Figure S8. Heatmap analysis of pre-monsoonal and monsoonal microbial class occurrence under two irrigation types.



Supplementary Figure S9. KEGG Metabolic pathway analysis with predominant biochemical modules as superimposed models of earlier reports. Our analysed pathways are highlighted with red lines in each diagram.

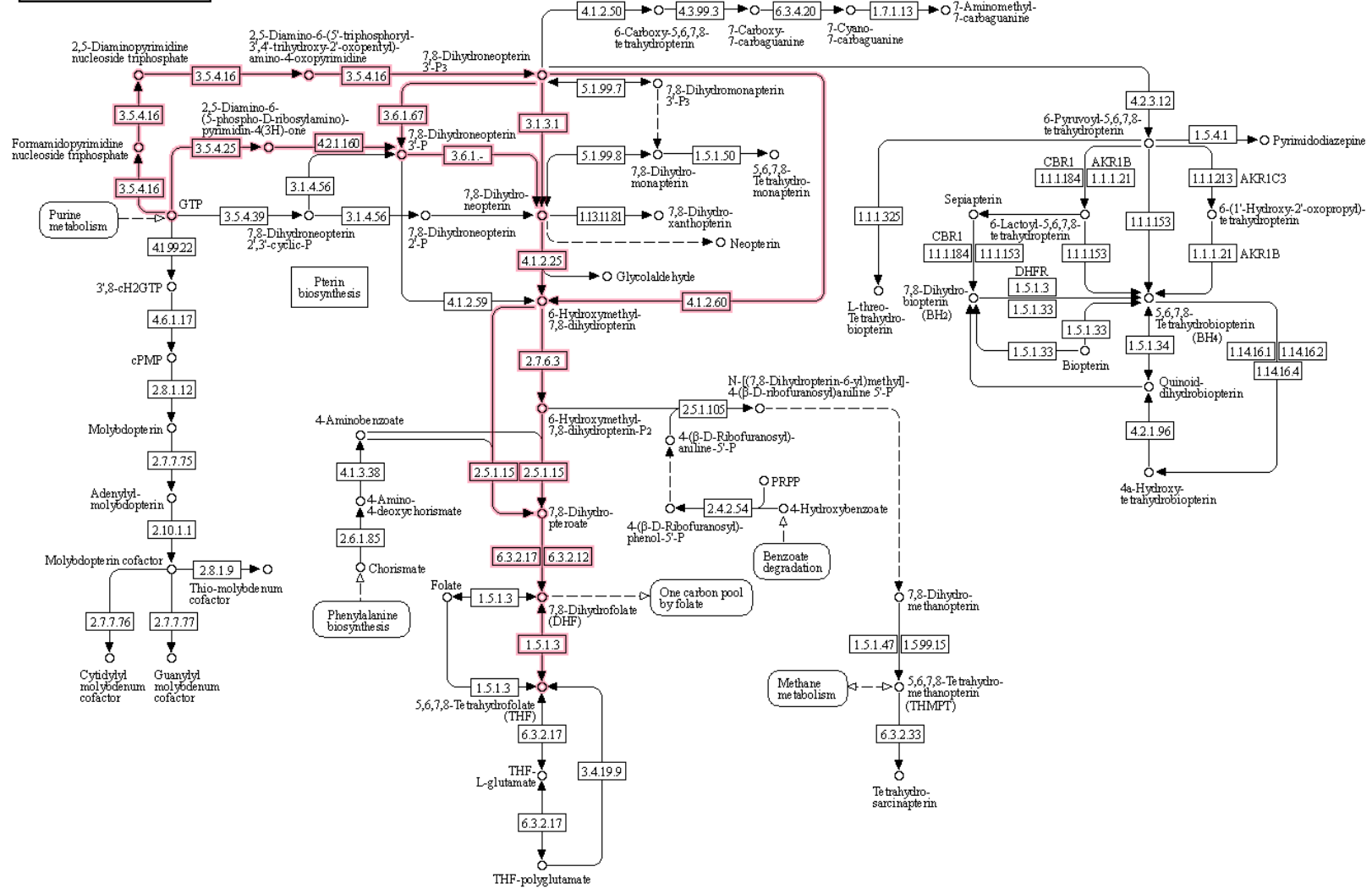


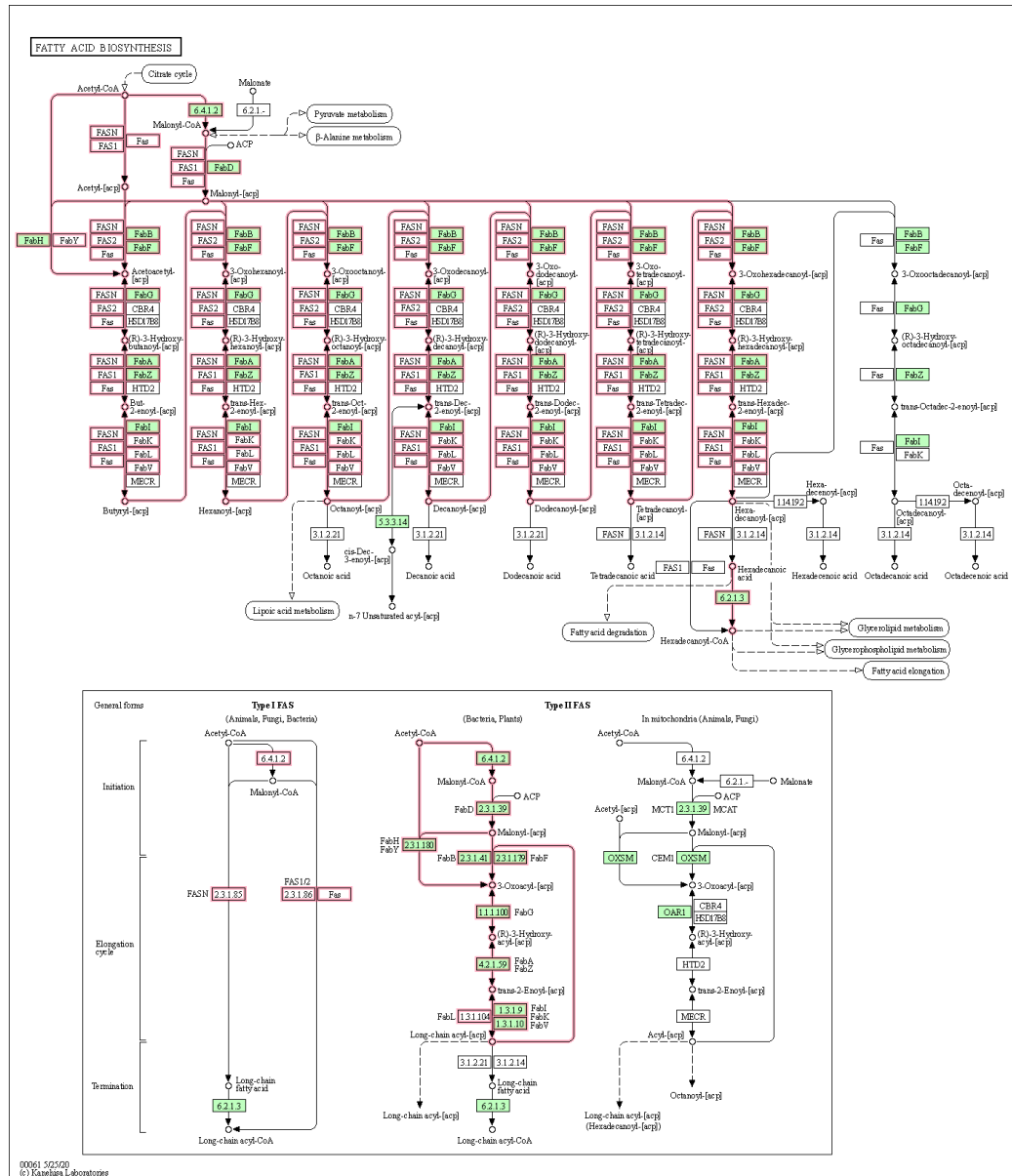
PYRIMIDINE METABOLISM



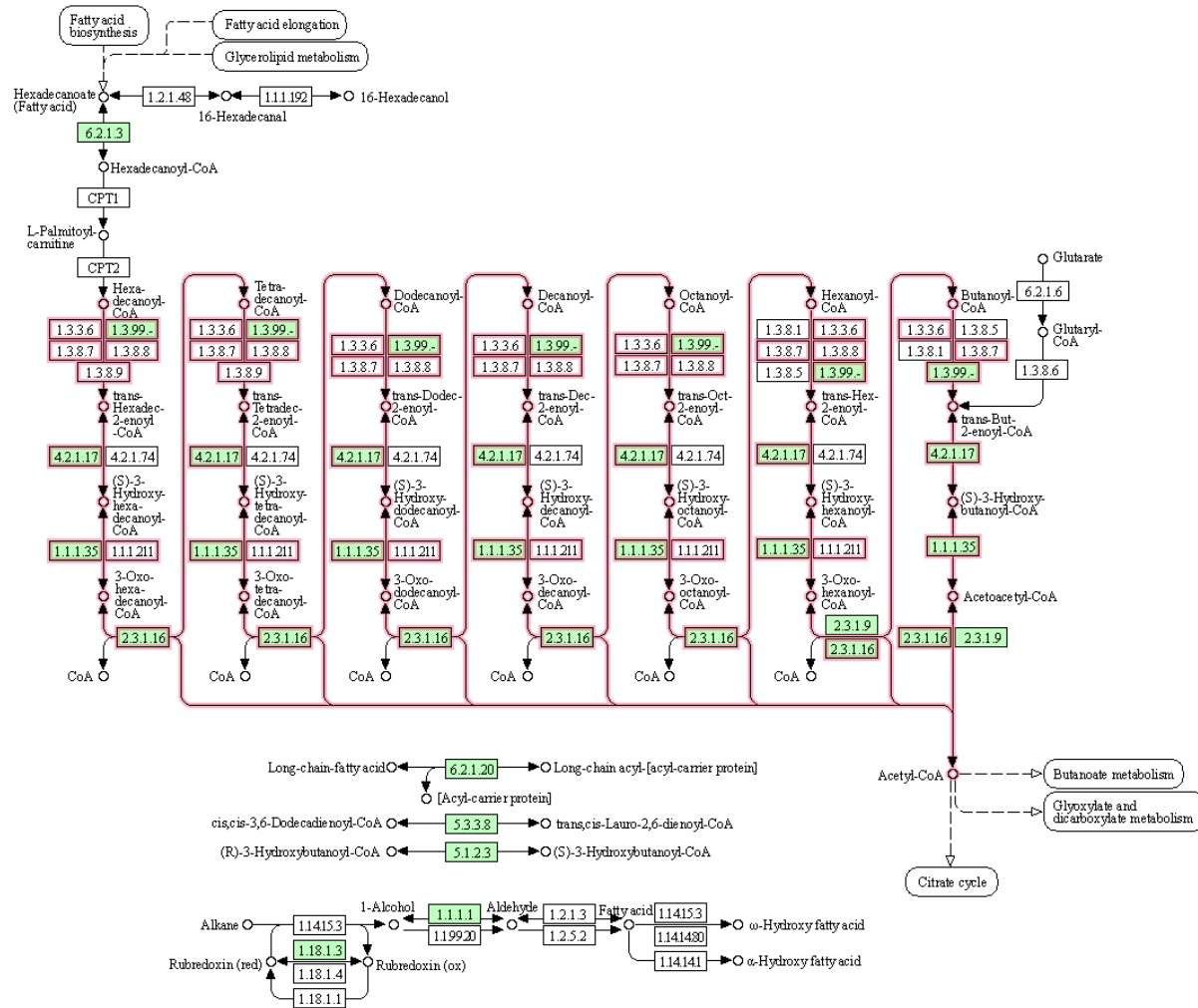
00240 4/26/21
© Kanehisa Laboratories

FOLATE BIOSYNTHESIS

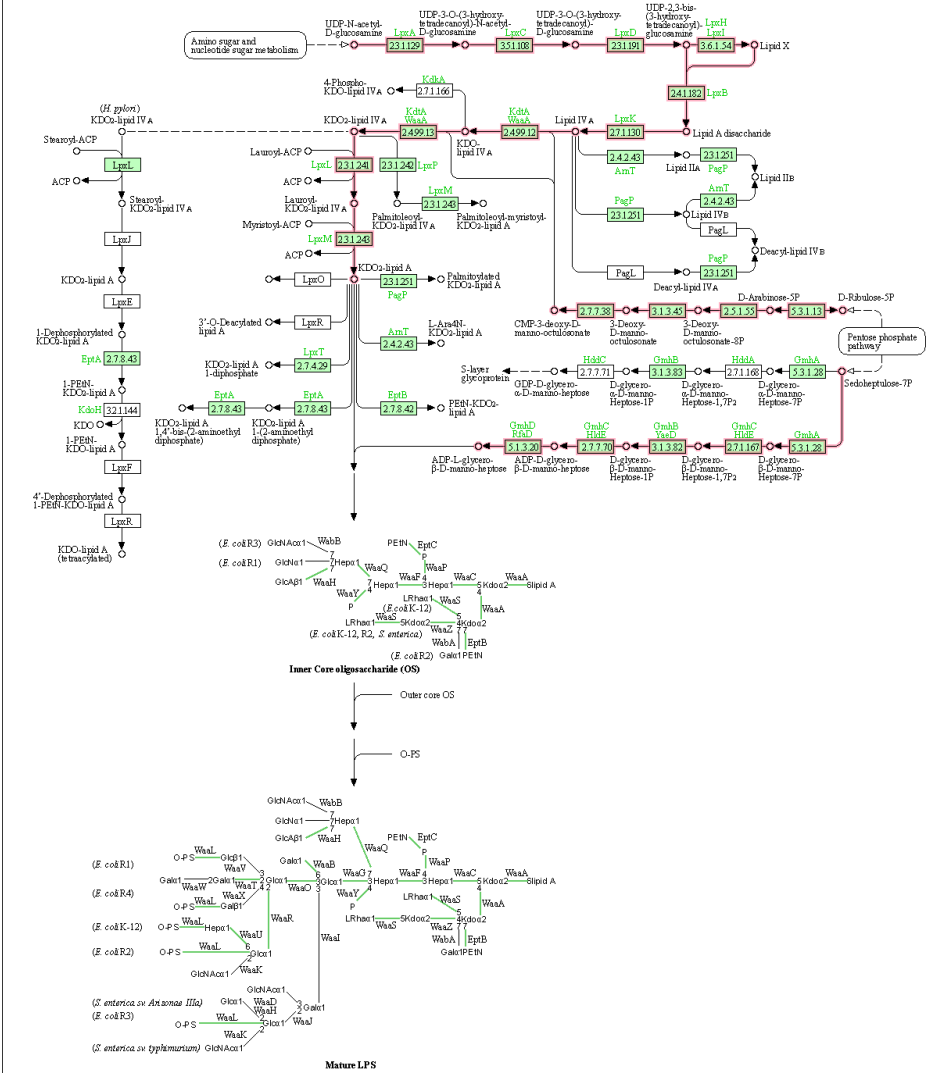




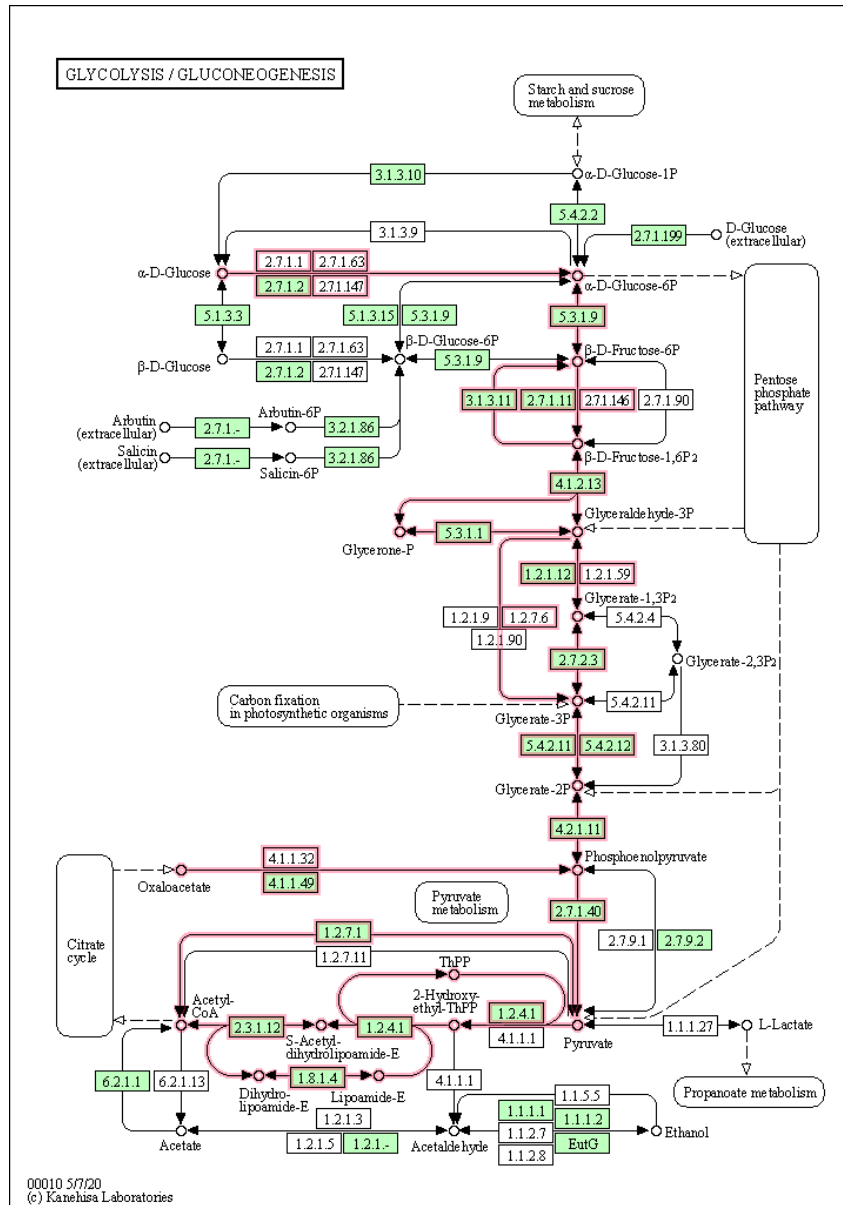
FATTY ACID DEGRADATION



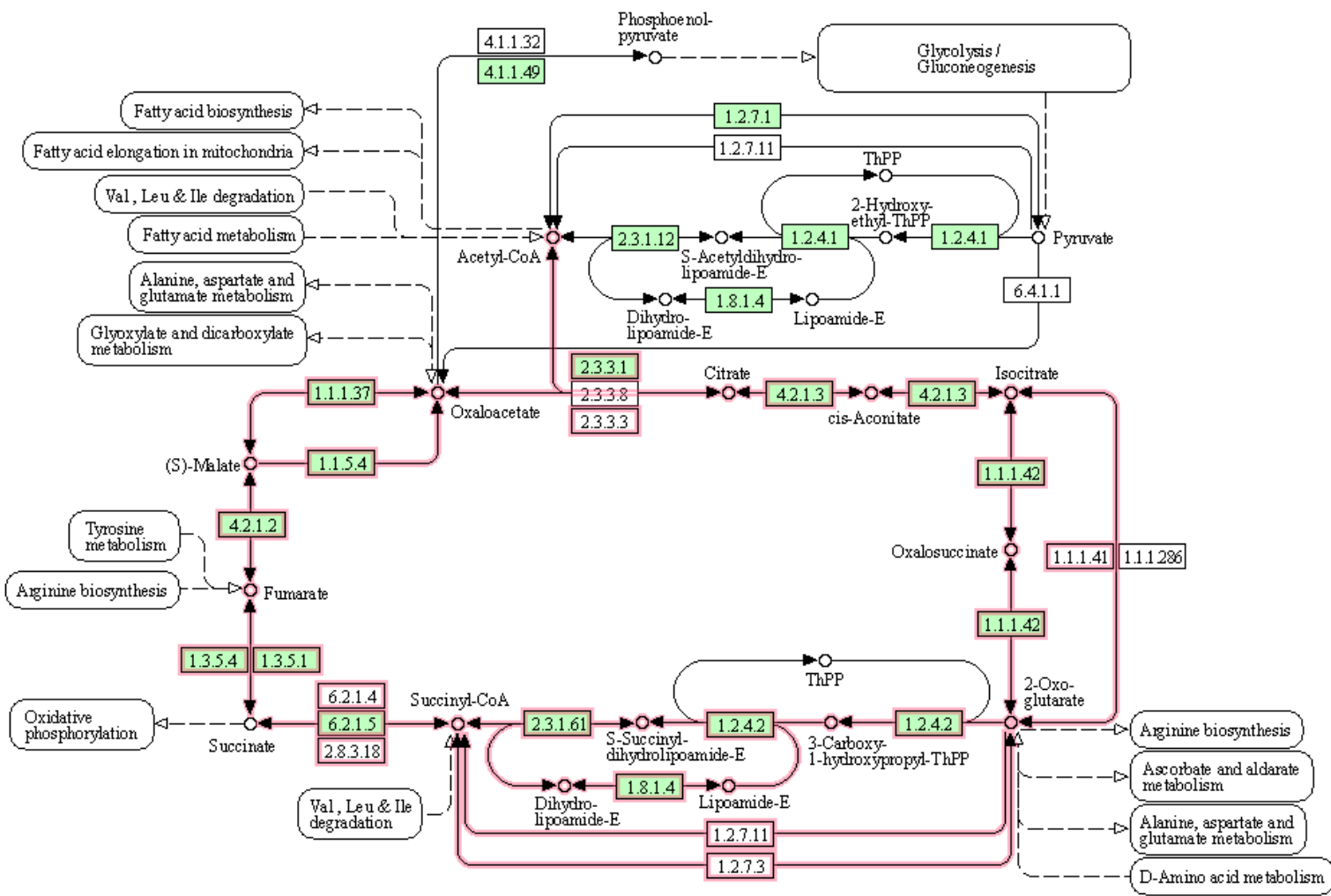
LIPOLYSACCHARIDE BIOSYNTHESIS



0805-0113201
© Galathea Laboratories

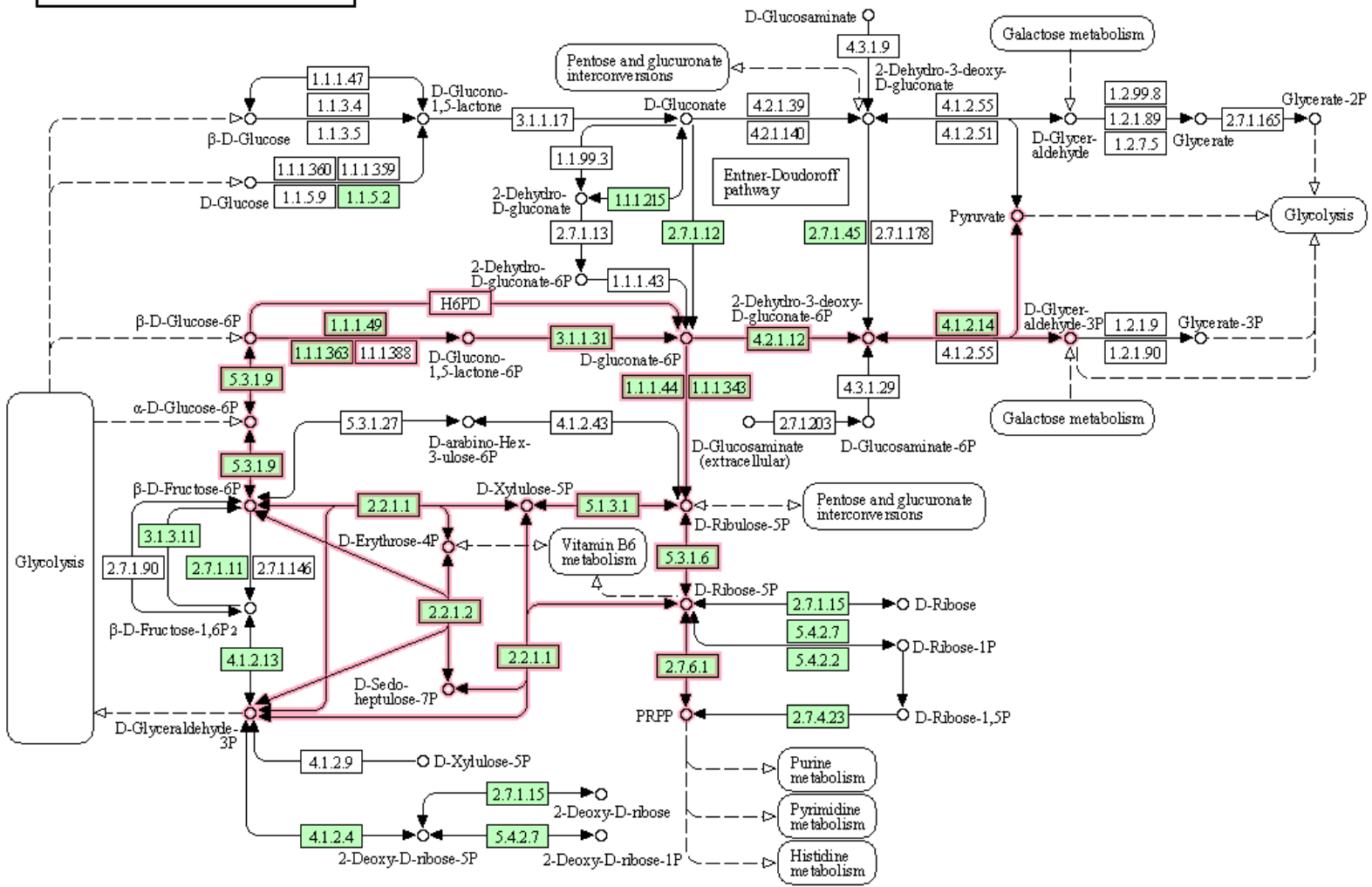


CITRATE CYCLE (TCA CYCLE)



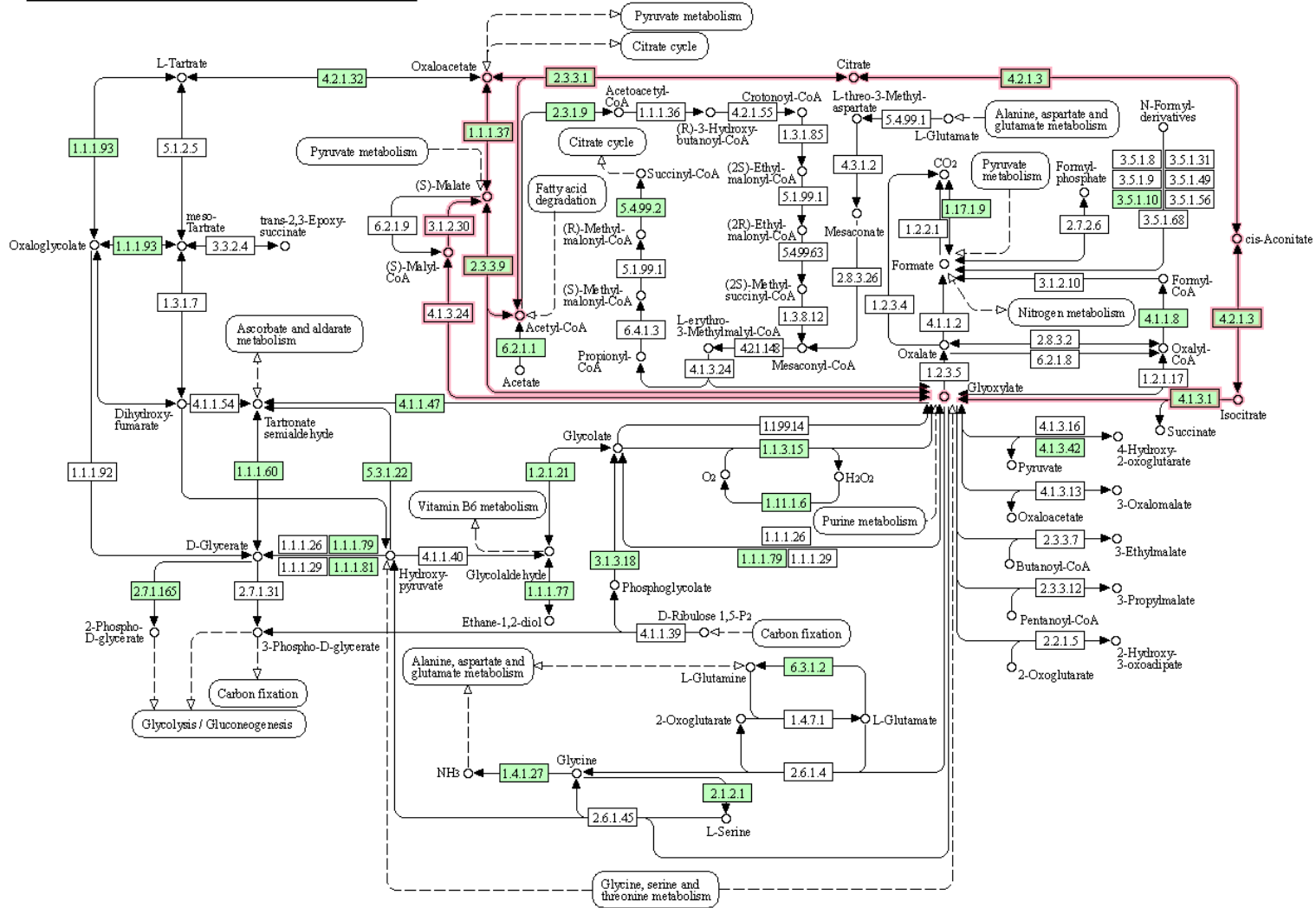
00020 9/22/21
 (c) Kanehisa Laboratories

PENTOSE PHOSPHATE PATHWAY

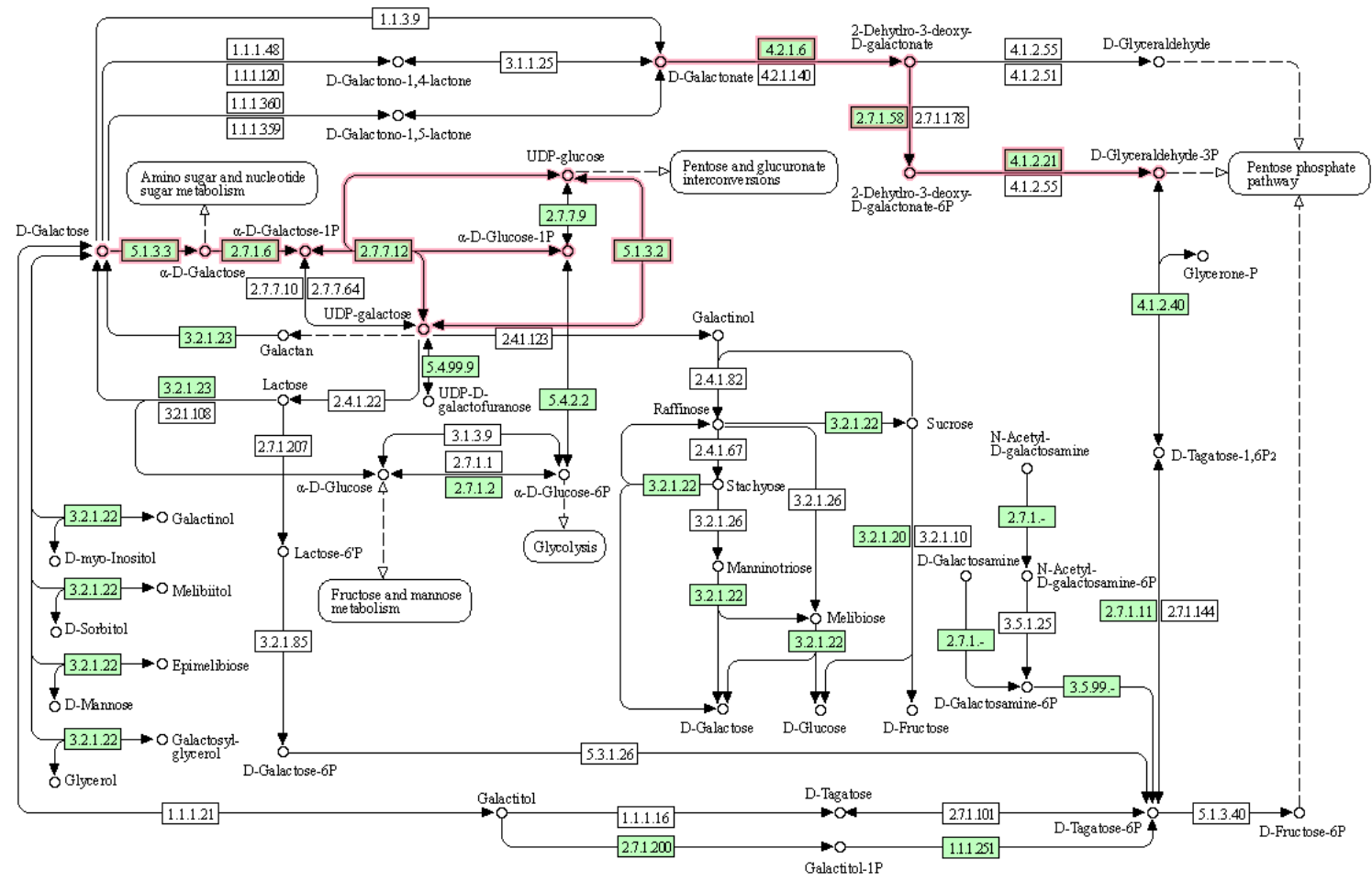


00030 9/9/20
(c) Kanehisa Laboratories

GLYOXYLATE AND DICARBOXYLATE METABOLISM

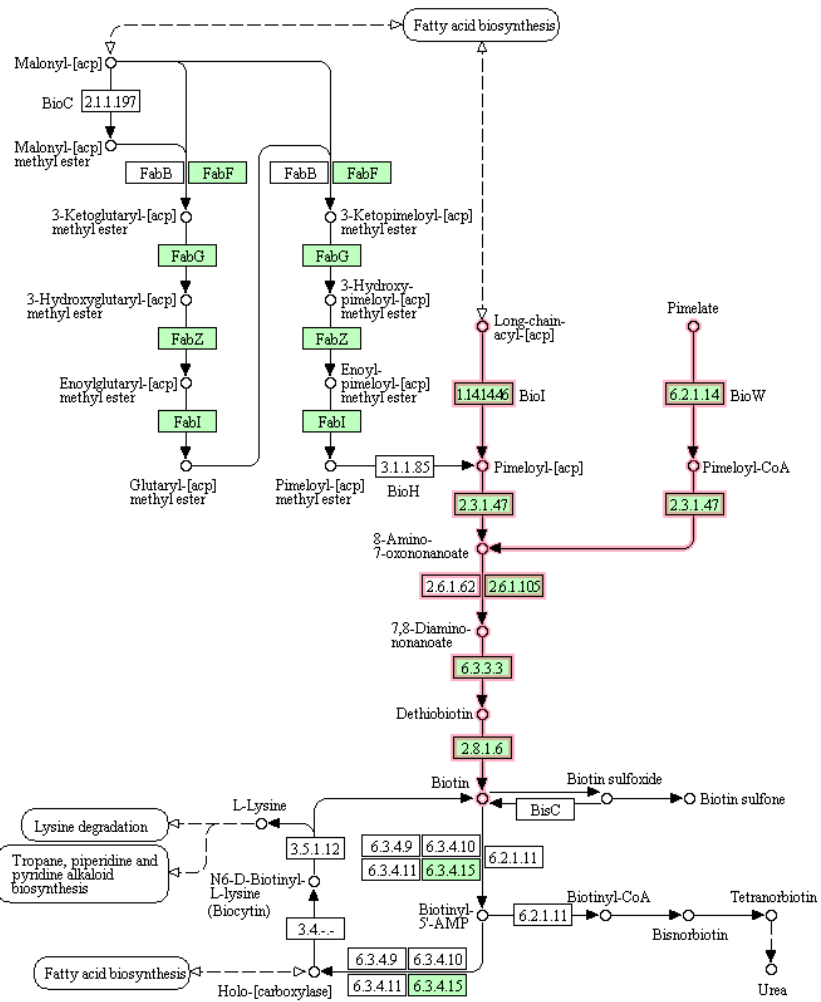


GALACTOSE METABOLISM



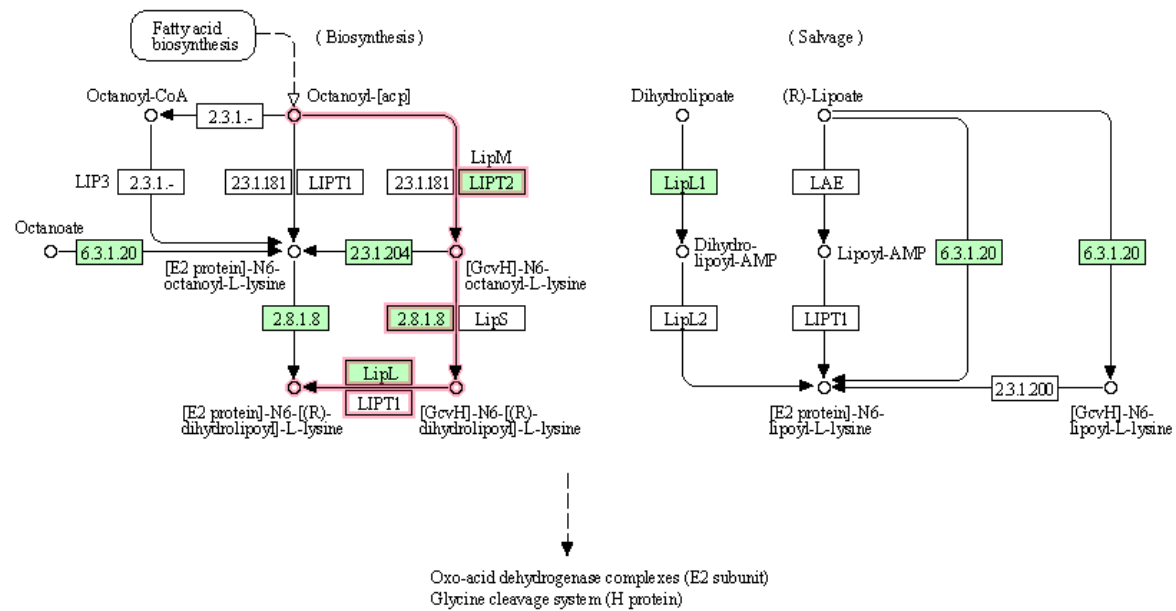
00052 8/20/21
 (c) Kanehisa Laboratories

BIOTIN METABOLISM



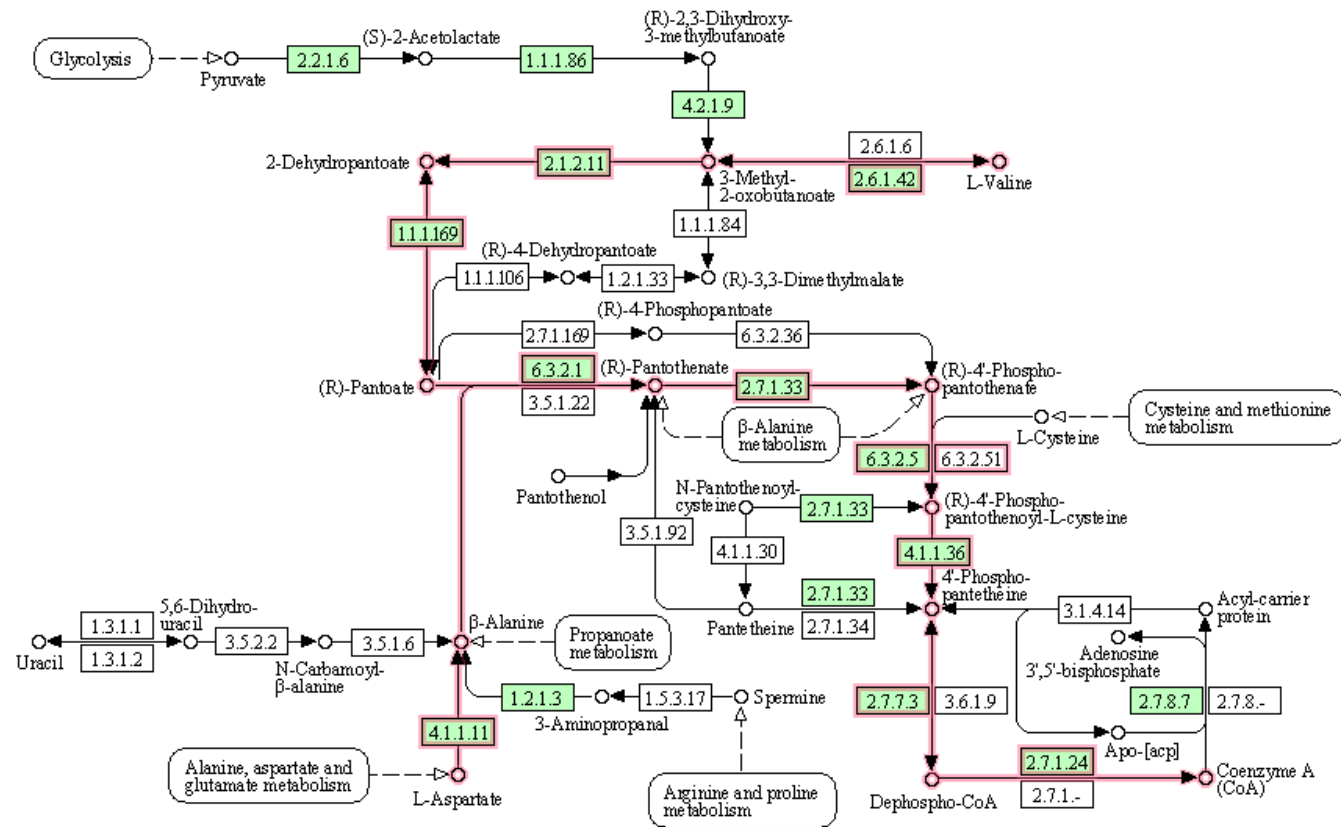
00780 7/19/17
 (c) Kanehisa Laboratories

LIPOIC ACID METABOLISM



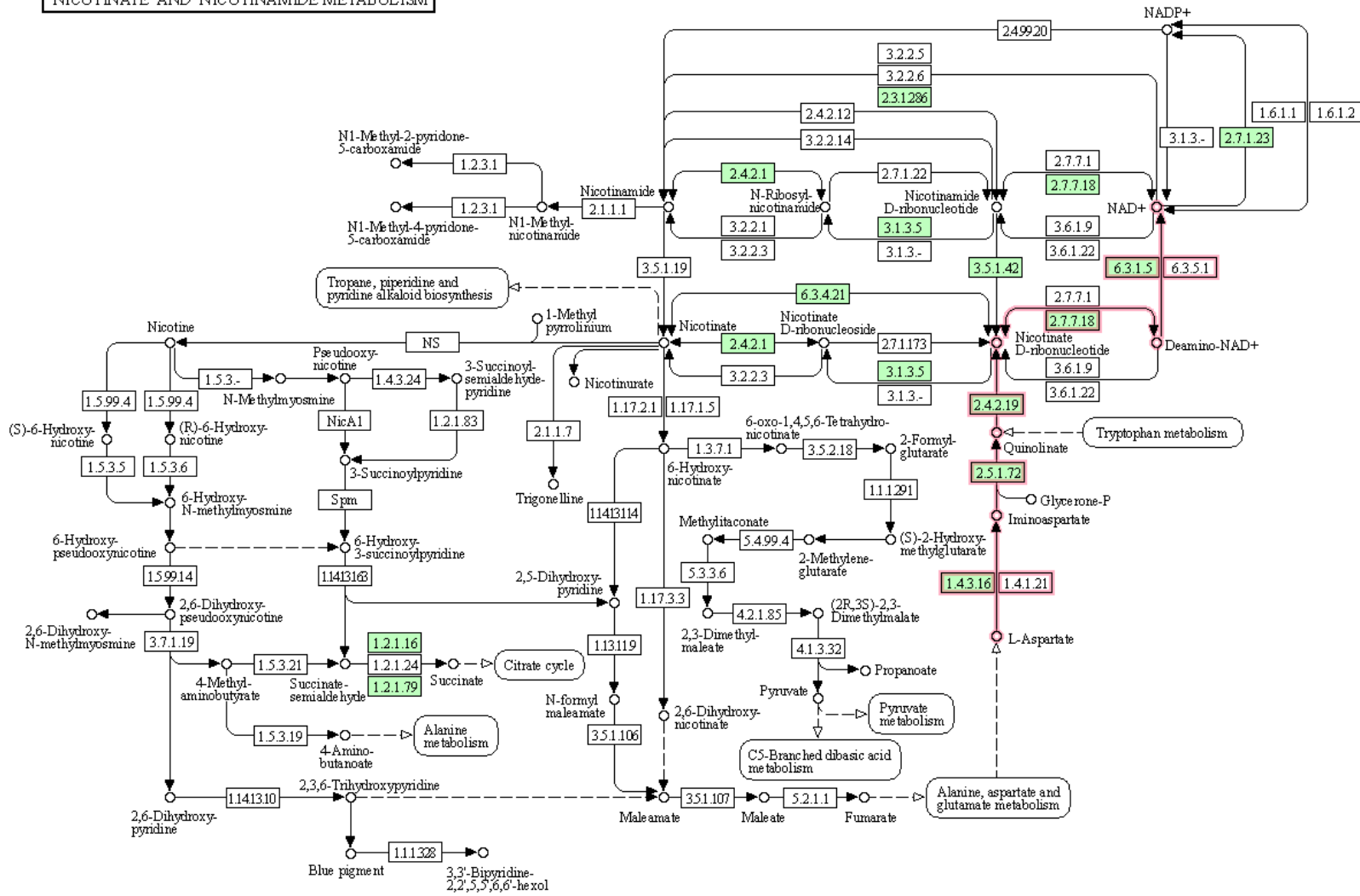
00785 1/27/21
 (c) Kanehisa Laboratories

PANTOTHENATE AND CoA BIOSYNTHESIS

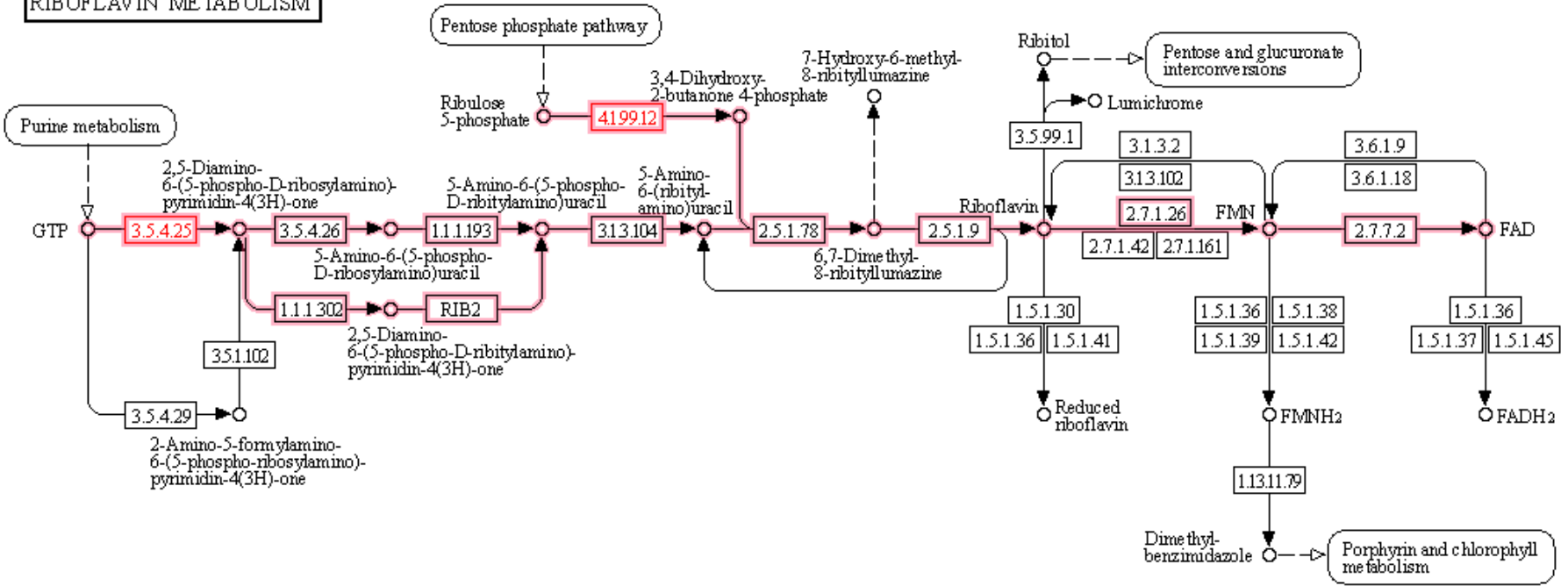


00770 7/7/20
 (c) Kanehisa Laboratories

NICOTINATE AND NICOTINAMIDE METABOLISM

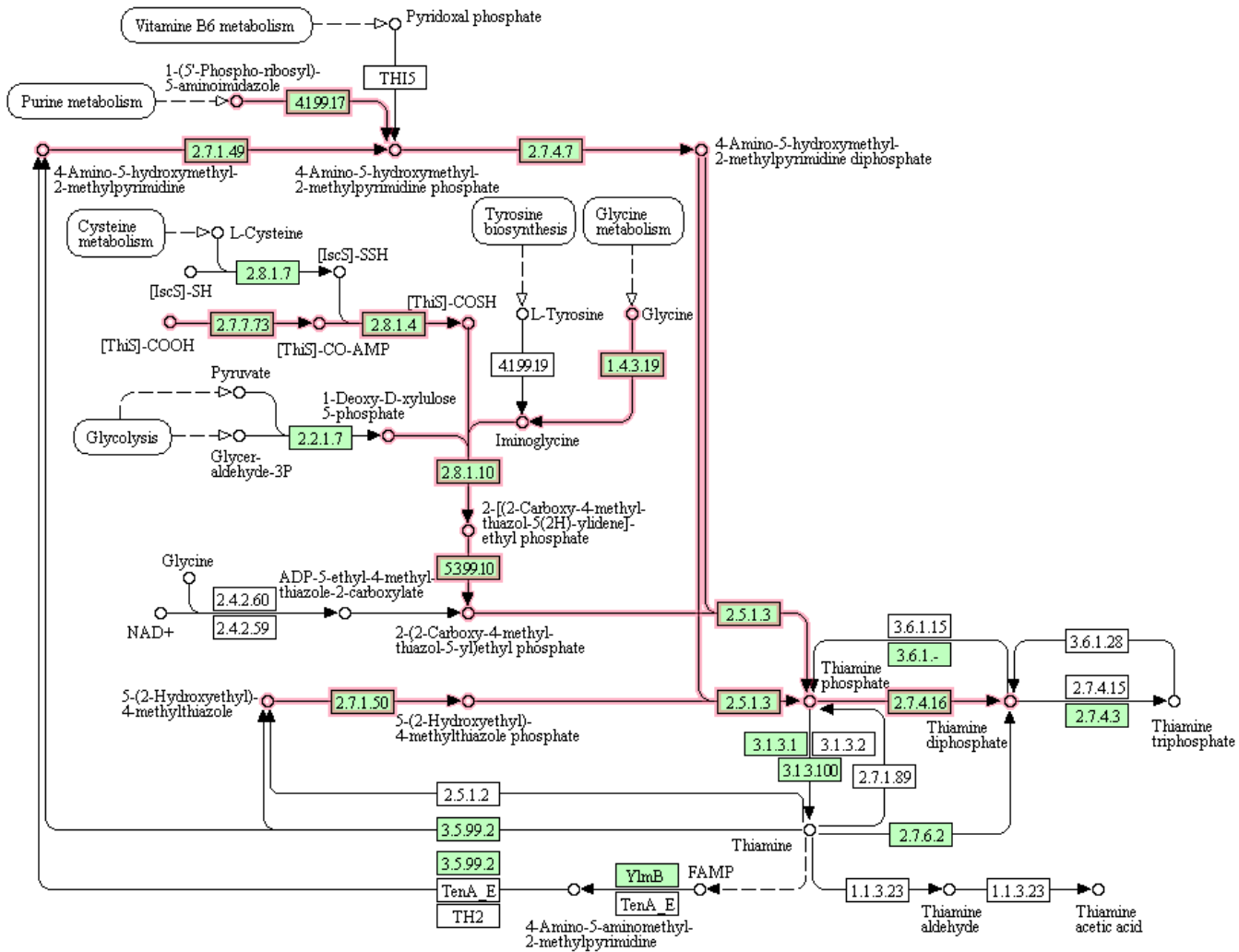


RIBOFLAVIN METABOLISM



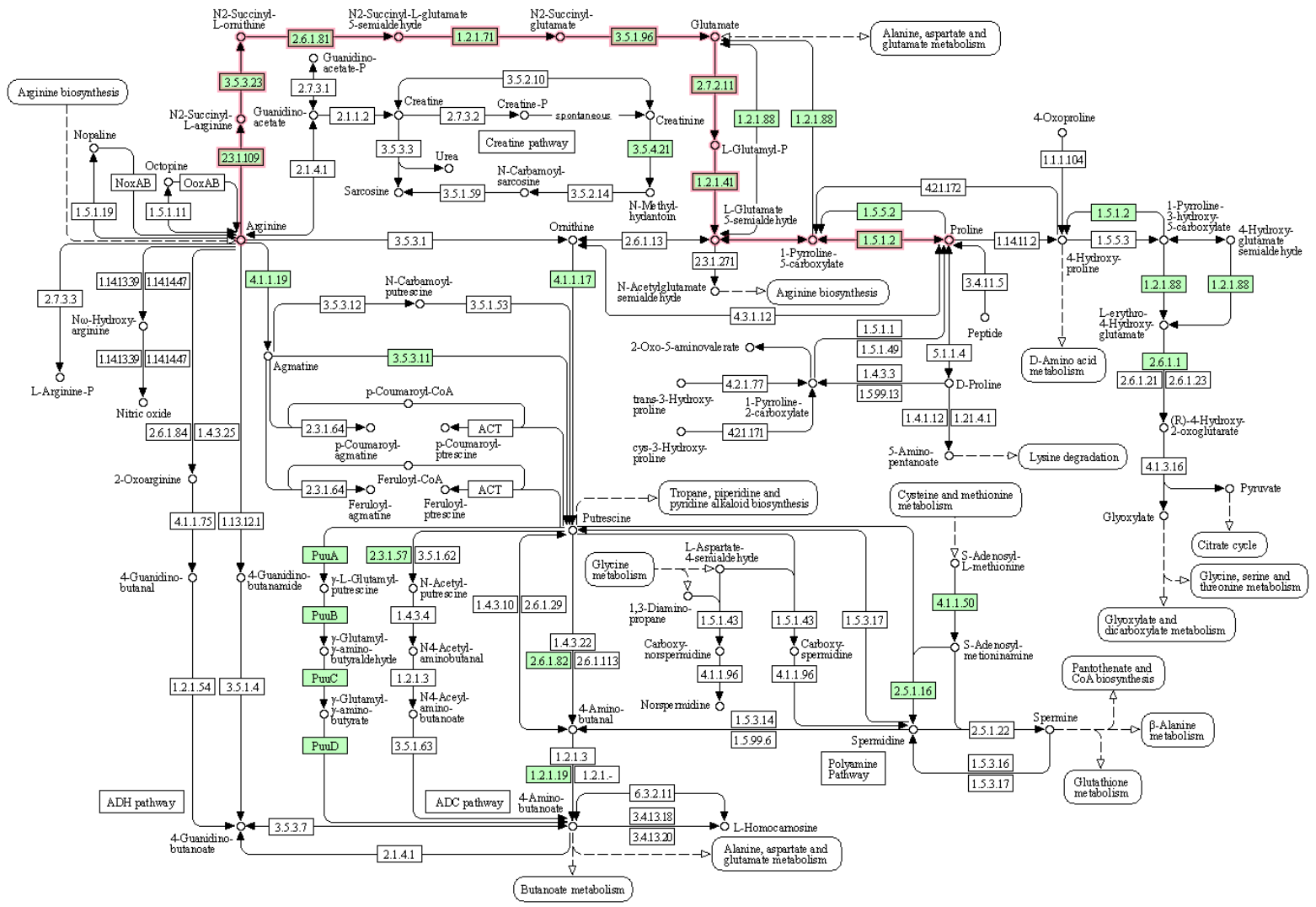
00740 4/11/19
 (c) Kanehisa Laboratories

THIAMINE METABOLISM



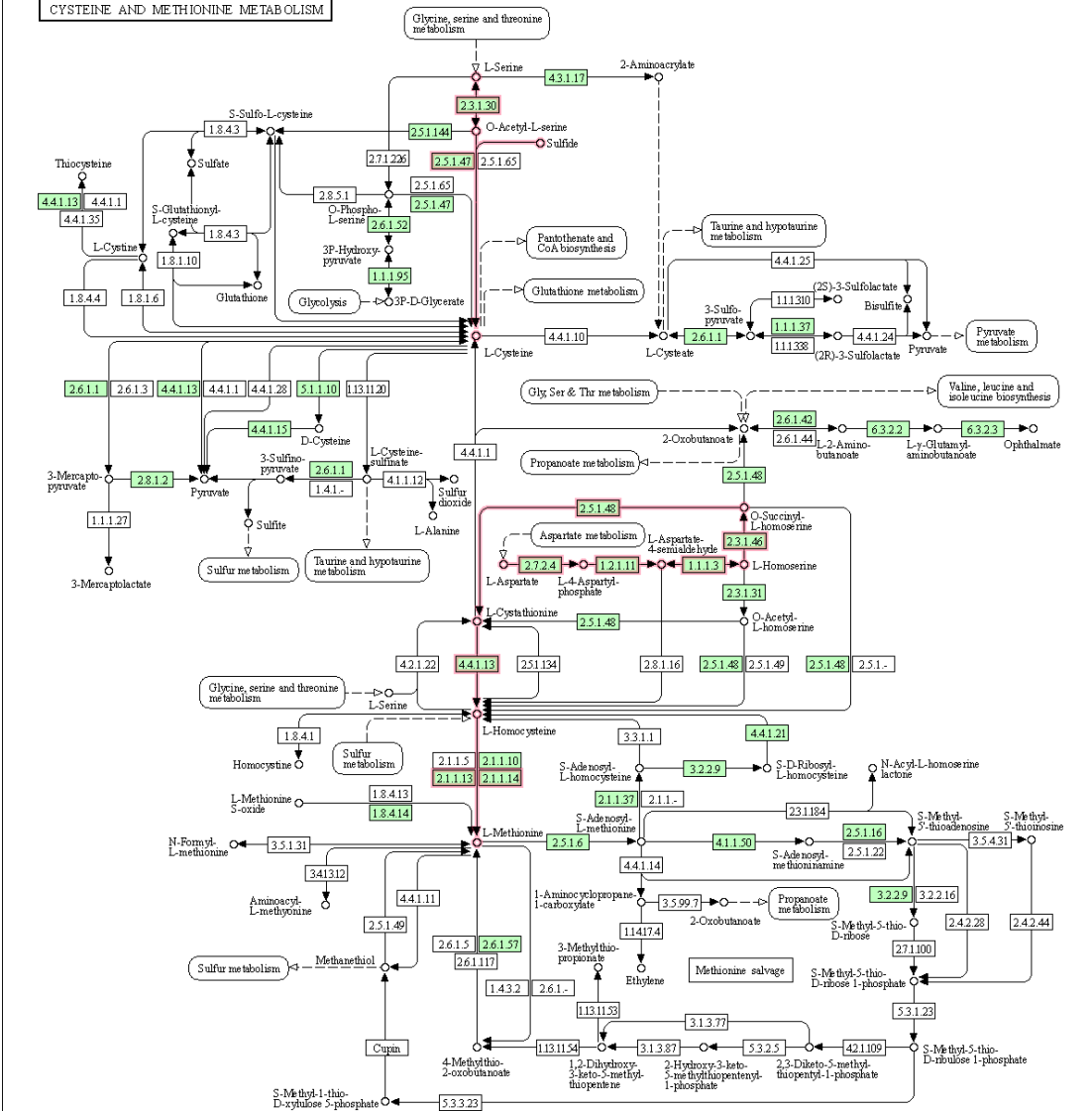
00730 8/21/18
 (c) Kanehisa Laboratories

ARGININE AND PROLINE METABOLISM



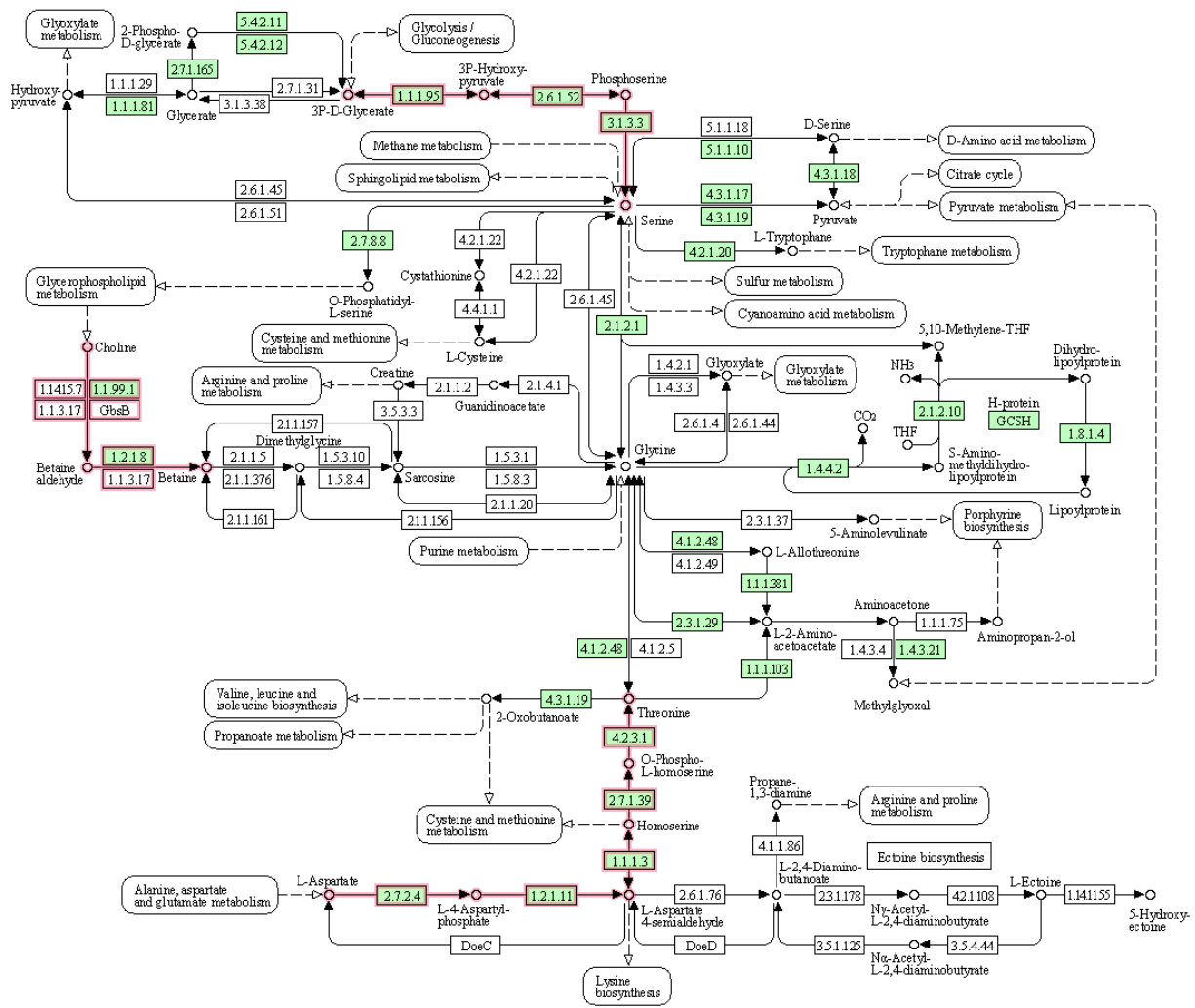
00330 10/11/21
 (c) Kanehisa Laboratories

CYSTEINE AND METHIONINE METABOLISM



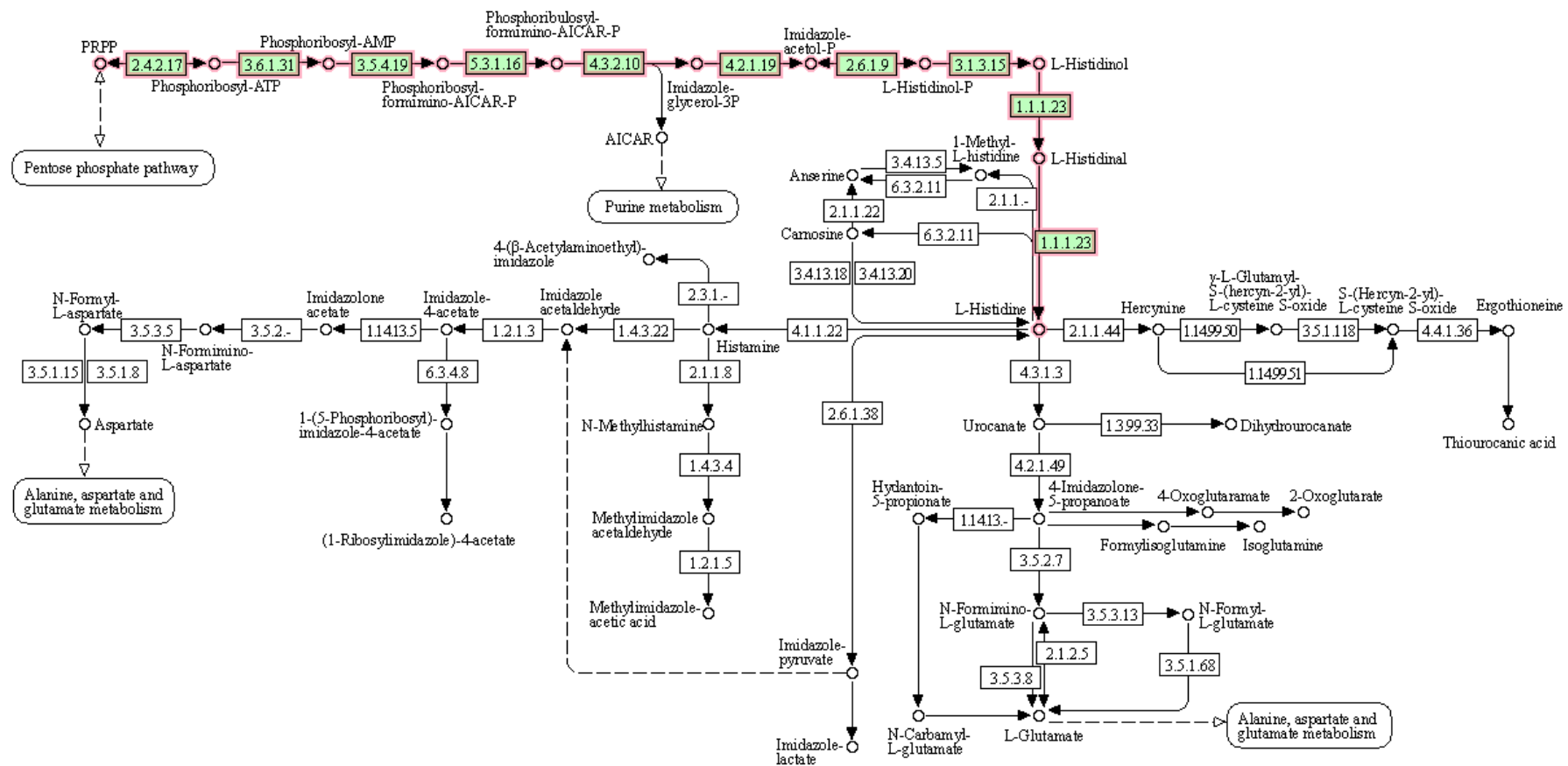
00270 5/26/21
© Kanehisa Laboratories

GLYCINE, SERINE AND THREONINE METABOLISM



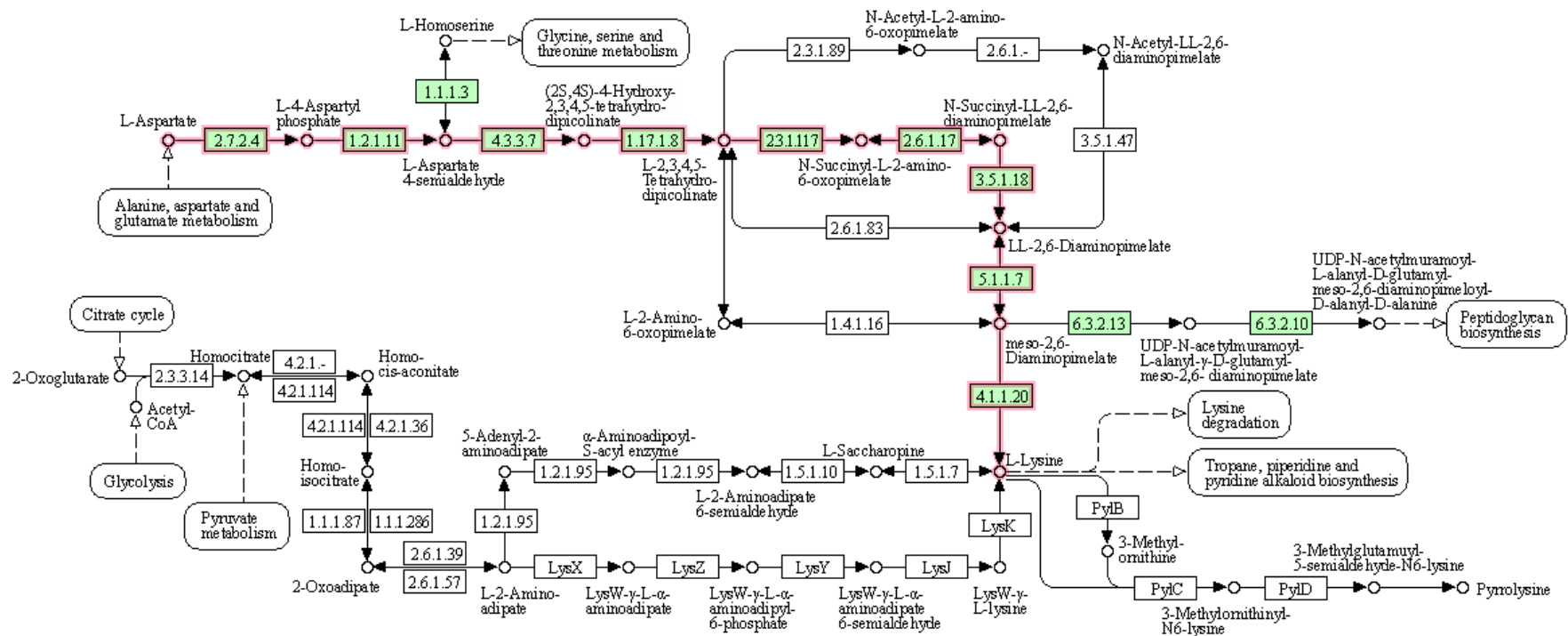
00260 10/11/21
 (c) Kanehisa Laboratories

HISTIDINE METABOLISM



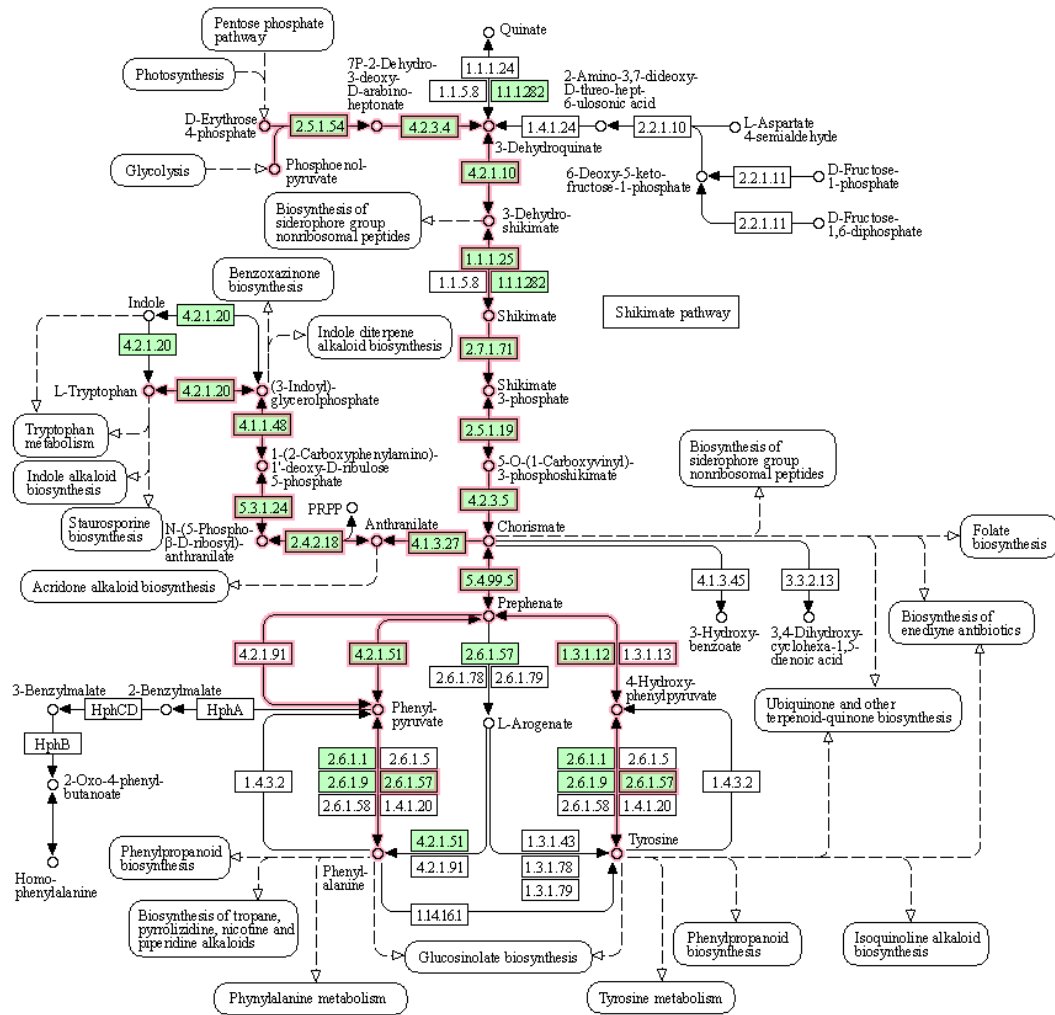
00340 10/5/18
 (c) Kanehisa Laboratories

LYSINE BIOSYNTHESIS

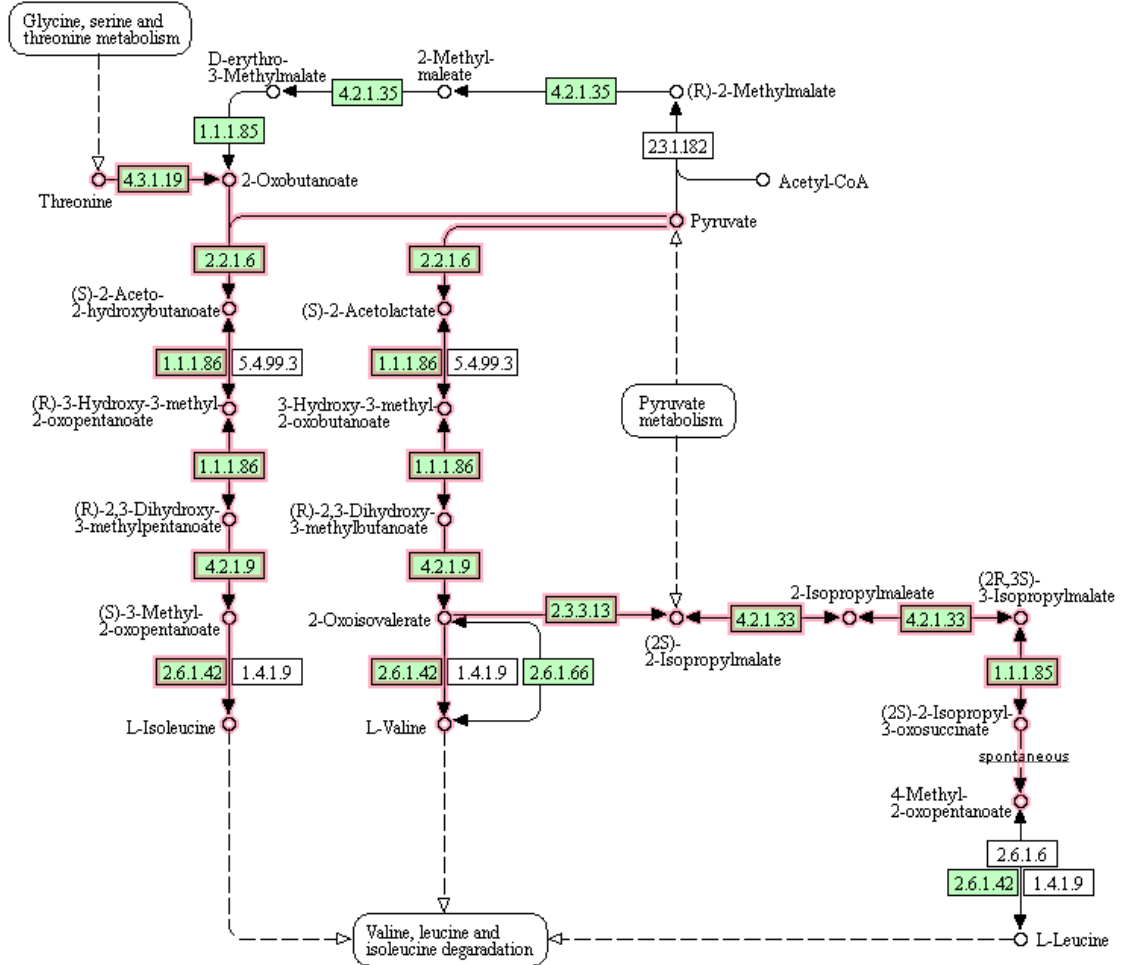


00300 5/25/21
(c) Kanehisa Laboratories

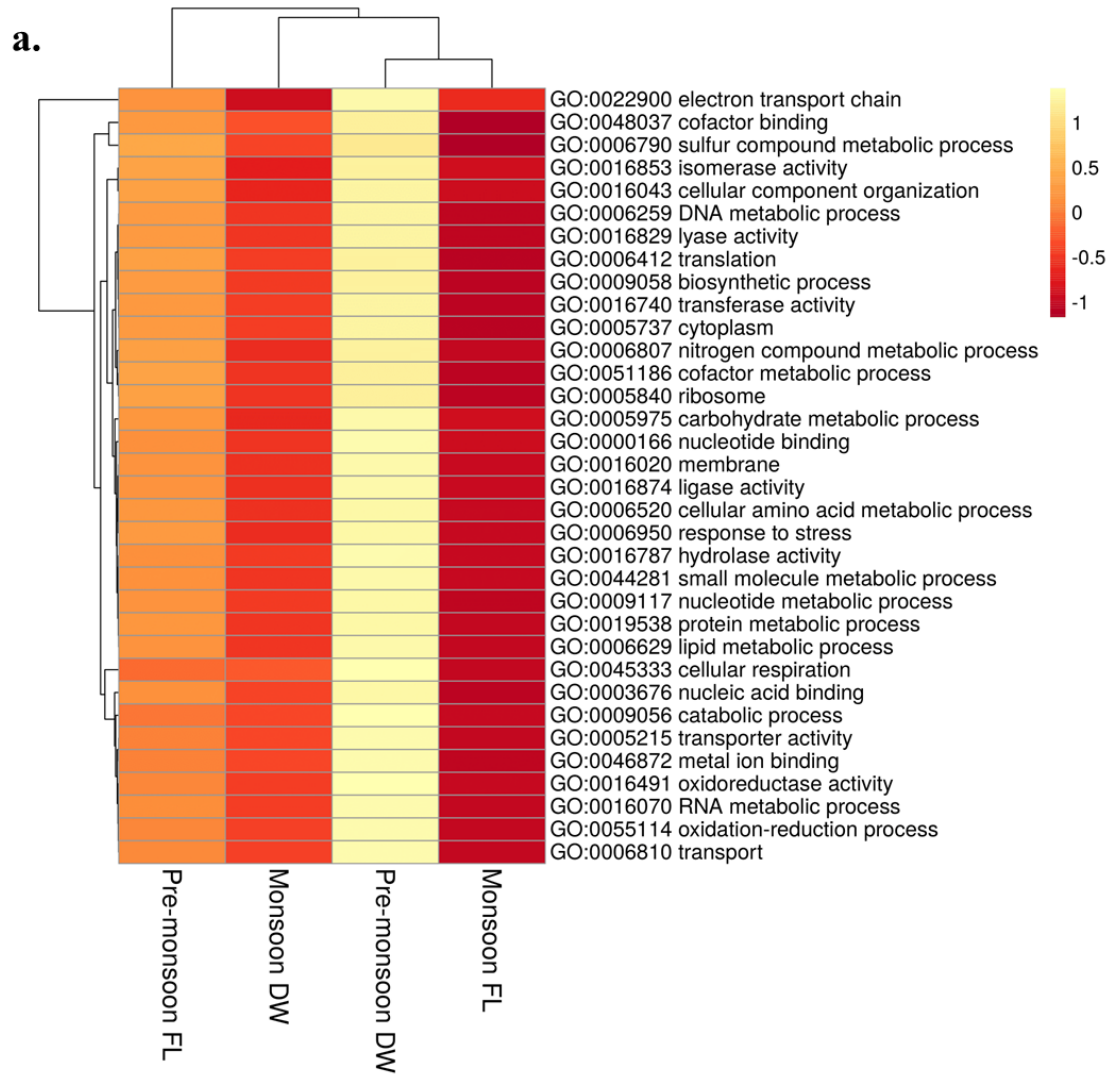
PHENYLALANINE, TYROSINE AND TRYPTOPHAN BIOSYNTHESIS



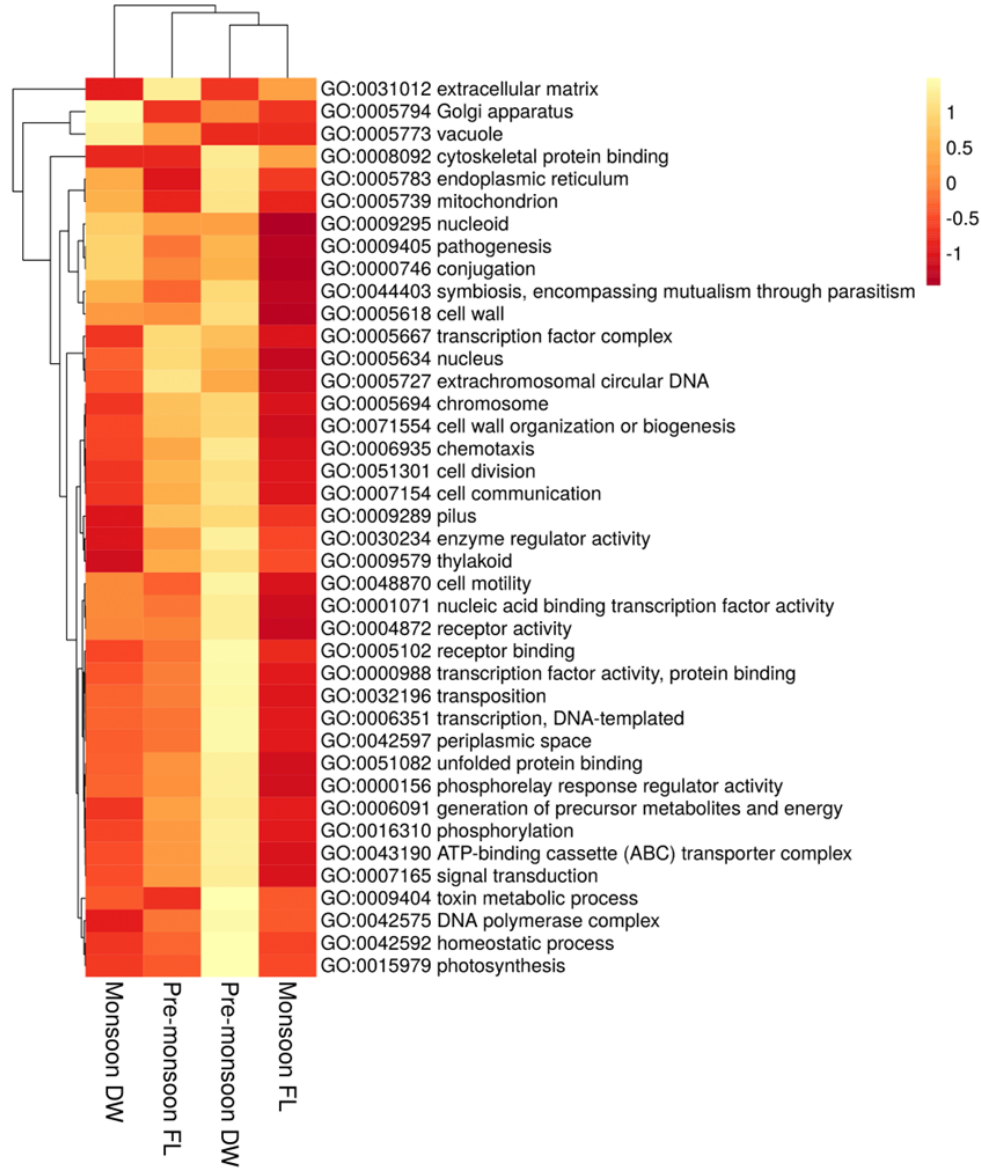
VALINE, LEUCINE AND ISOLEUCINE BIOSYNTHESIS



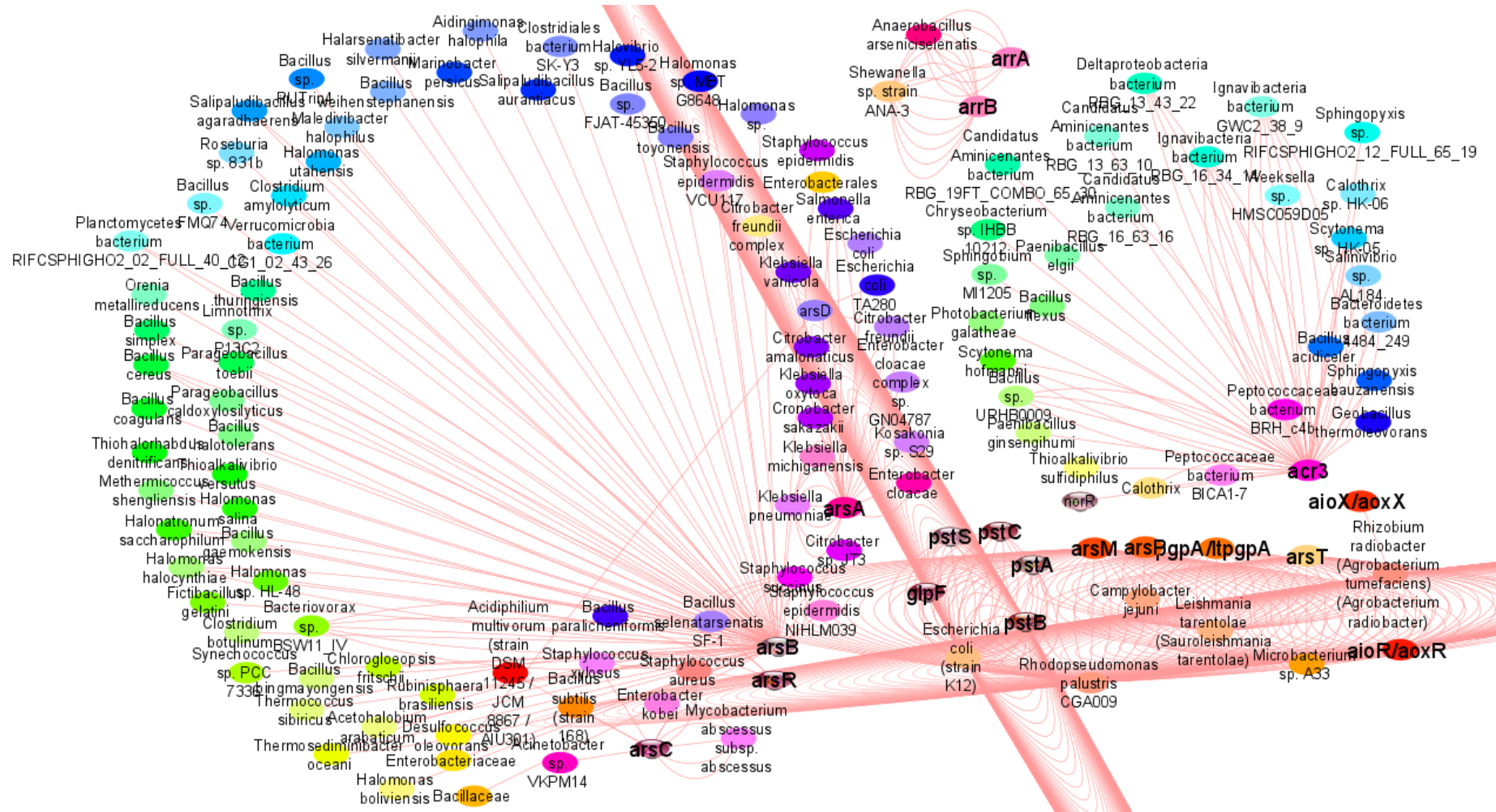
Supplementary Figure S10. Gene Ontology (GO) profiling of dominant microbial metabolisms with higher frequency (a) and lower frequency (b) activities.

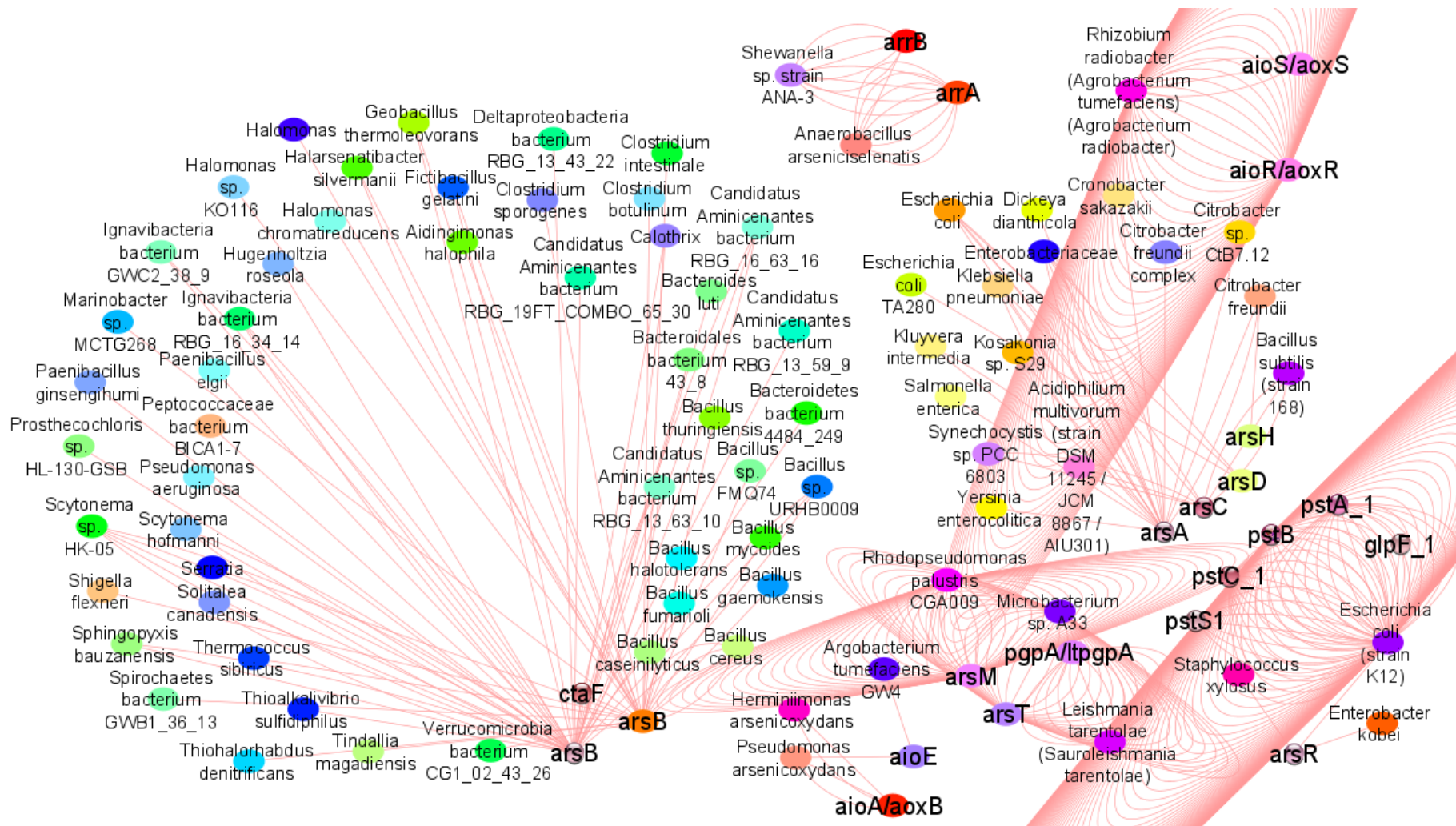


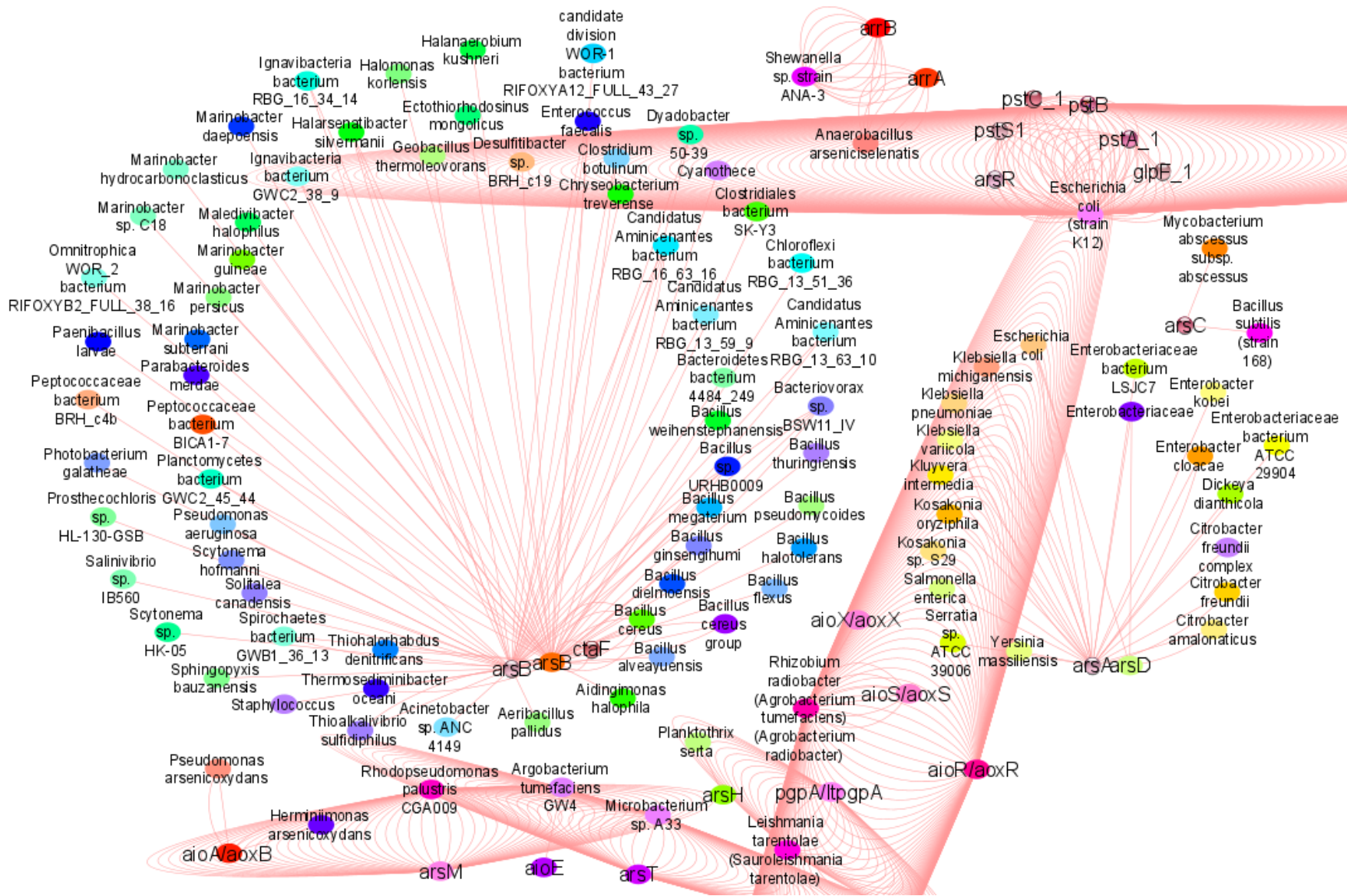
b.

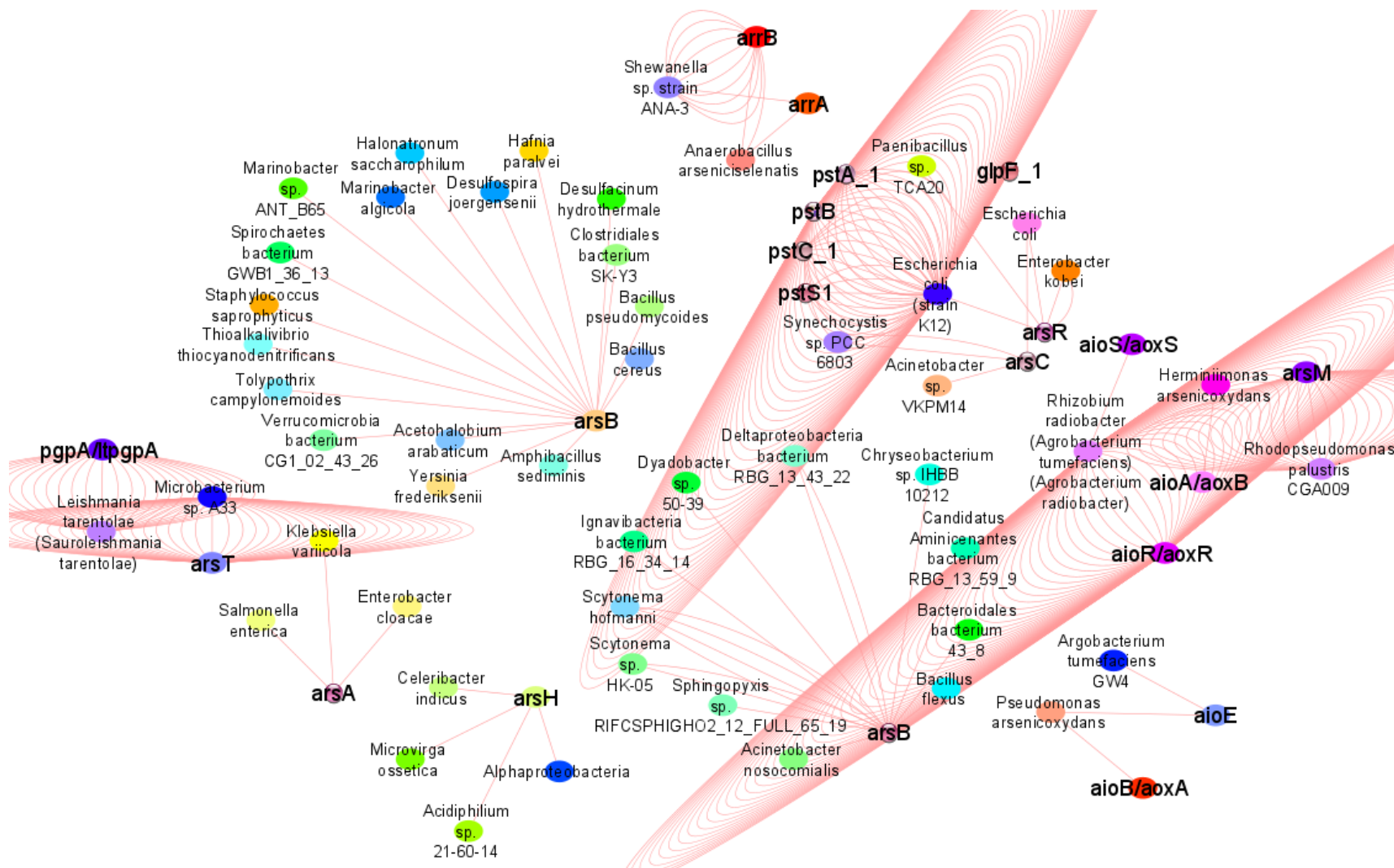


Supplementary Figure S11. Arsenic responsive gene-dependent dominant microbial network analysis in pre-monsoonal dry-wet/flooded (a,b) and monsoonal dry-wet/flooded (c,d) phases respectively.



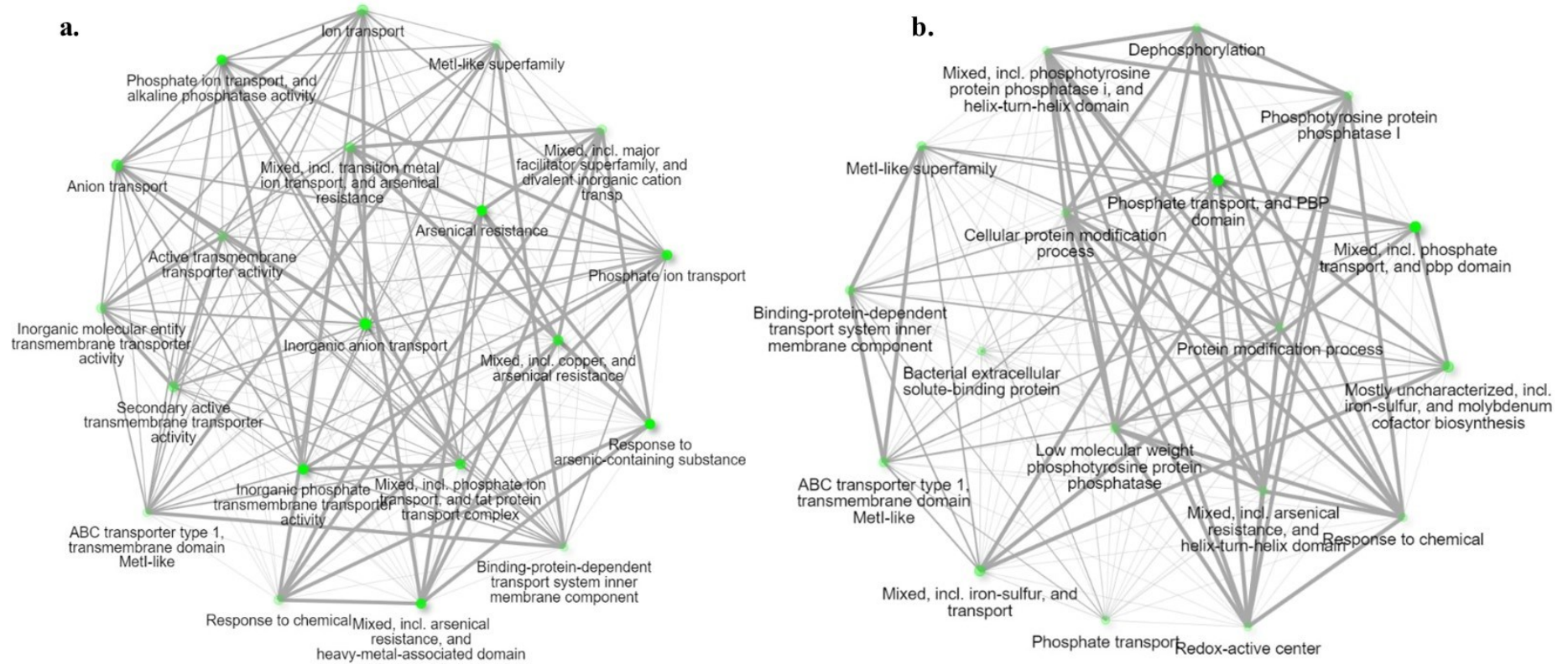




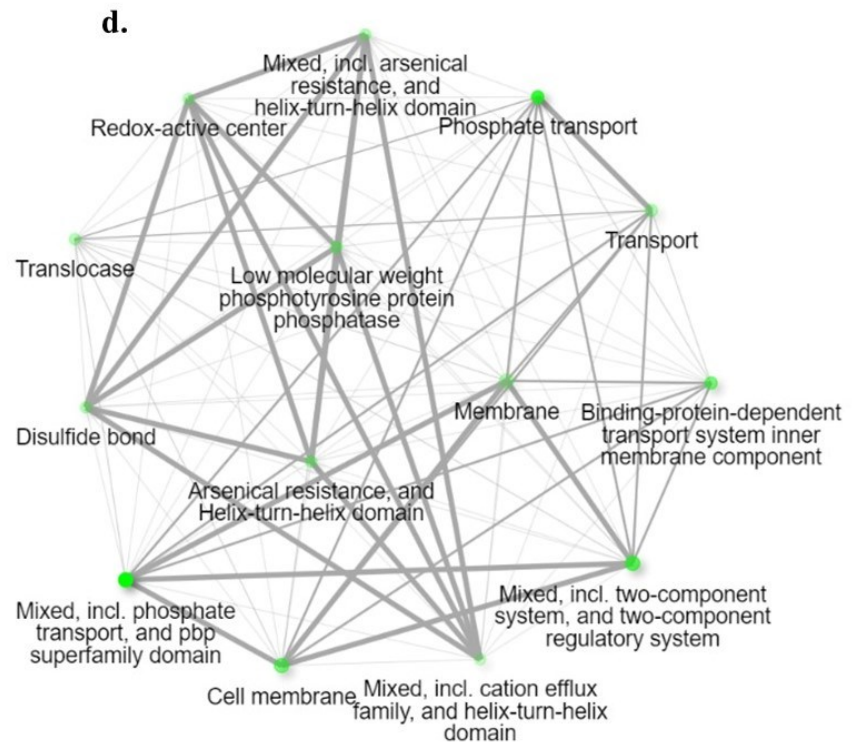
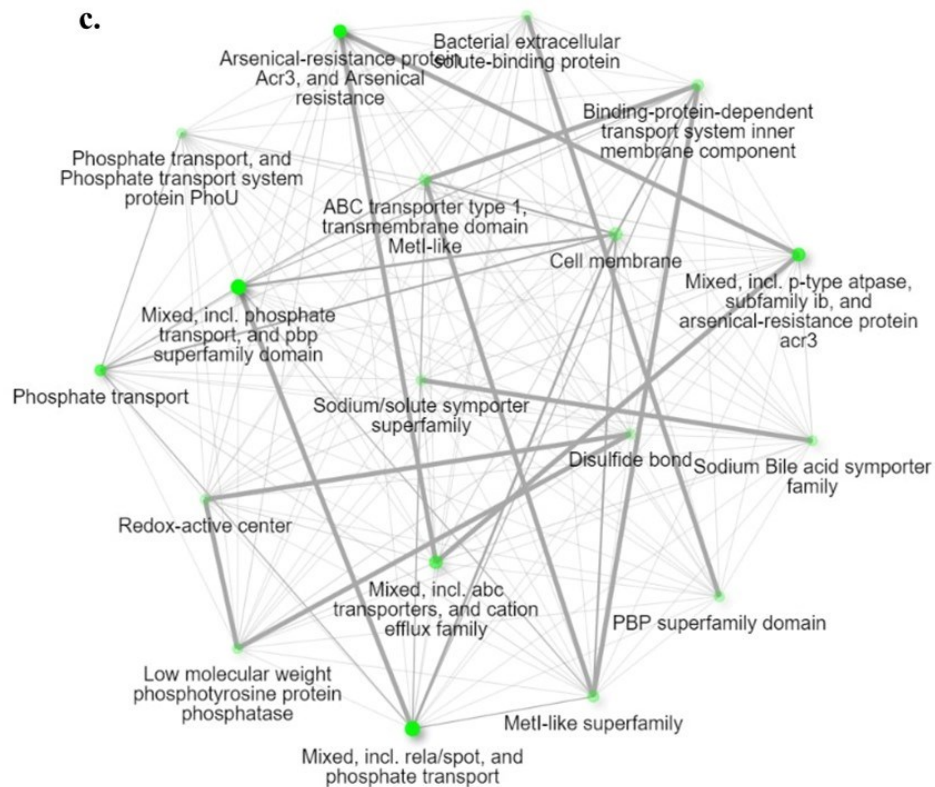


Soil microbial communities actively express different arsenic-responsive and related genes that also influence their diversity, inter-connectivity and networking pattern as presented in Figure 5a-d. Pre-monsoonal and monsoonal soil microbes under dry-wet and flooded conditions are presented here. Data are representative of mean read counts from 24 sampling sites.

Supplementary Figure S12. Network relationship of As-responsive and related genes under pre-monsoonal dry-wet and flooded soils (a-b) and monsoonal dry-wet and flooded soils (c-d). The genes are named within the figure.



arrB, aioA, arrA, arsR, arsB, arsC, arsA, arsR, arsD, arsH, aioE, arsT, arrB, pstS, pstC, pstB, pstA, pgpA, arsP, arsM, aioX, aioS, aioR, aioA, aoxX, aoxS, aoxR, aoxB, ltpgpA, aoxB



arrB, aioA, arrA, arsR, arsB, arsC, arsA, arsR, arsD, arsH, aioE, arsT, arrB, pstS, pstC, pstB, pstA, pgpA, arsP, arsM, aioX, aioS, aioR, aioA, aoxX, aoxS, aoxR, aoxB, ltpgpA, aoxB

Supplementary Table S1. Standard reference material values as analyzed for quality assurance.

SRM Name	As (mg kg ⁻¹)	SiO ₂ (%)
	MESS3 Obtained data	102.4
Certified data	107	57.76
RD	4.492187	1.09072
NIST 2711a Obtained data	22.47	66.72
Certified data	21.2	67.17
RD	-5.65198	0.669942
JSD3 Obtained data	2.37	60.05
Certified data	2.42	60.78
RD	2.109705	1.201053
SDC1 Obtained data	25	65.61
Certified data	25.7	65.8
RD	2.8	0.288754

NIST1568c	As (mg kg ⁻¹)
	Obtained data
Certified data	0.359
RD	3.064

Supplementary Table S2. Soil texture analysis by ASTM sieve method of all 24 sites within a radius of 1km from the Ganga bed.

Gangetic divisions	Sites	Sand%	Silt%	Clay%
Upper Ganga (Uttarakhand)	Site 1	24.00±1.6	26.00±1.9	50.00±2.3
	Site 2	23.00±1.8	25.00±2.6	52.00±1.5
	Site 3	26.00±1.6	26.00±2.3	48.00±1.6
	Site 4	24.00±1.9	27.00±1.5	49.00±1.9
Upper-middle Ganga (Uttar Pradesh)	Site 5	22.00±2.1	23.00±1.6	55.00±2.3
	Site 6	23.00±2.5	24.00±1.2	53.00±2.1
	Site 7	25.00±1.7	28.00±1.3	47.00±2.2
	Site 8	24.00±2.3	26.00±2.4	50.00±1.5
Middle Ganga (Uttar Pradesh)	Site 9	21.00±2.1	24.00±2.6	55.00±2.6
	Site 10	22.00±1.5	27.00±2.3	51.00±1.7
	Site 11	21.00±2.8	26.00±2.7	53.00±2.1
	Site 12	23.00±1.9	23.00±1.9	54.00±2.4
Middle Ganga (Bihar)	Site 13	24.00±2.6	21.00±1.8	55.00±1.9
	Site 14	21.00±2.4	28.00±1.2	51.00±1.6
	Site 15	21.00±1.7	24.00±2.5	55.00±2.5
	Site 16	20.00±2.3	25.00±2.3	55.00±2.4
Middle-lower Ganga (West Bengal)	Site 17	19.00±1.8	29.00±2.2	52.00±2.3
	Site 18	16.00±2.5	25.00±1.8	59.00±1.8
	Site 19	19.00±1.1	28.00±1.4	53.00±1.2
	Site 20	18.00±1.9	27.00±1.6	55.00±2.9
Lower Ganga (West Bengal)	Site 21	17.00±1.7	26.00±2.1	57.00±2.4
	Site 22	19.00±2.3	24.00±1.8	57.00±2.5
	Site 23	18.00±2.2	28.00±1.6	54.00±1.8
	Site 24	17.00±2.8	25.00±2.1	58.00±2.4

Supplementary Table S3. Alpha and Beta diversity indices of soil microbiome under different field setups and seasonal changes.

Gangetic Division 1 (Uttarakhand)				Gangetic Division 2 (Uttar Pradesh)				Gangetic Division 3 (Uttar Pradesh)			
Field samples	Alpha diversity indices			Field samples	Alpha diversity indices			Field samples	Alpha diversity indices		
Pre-monsoon	Shannon	Simpson	Chao1	Pre-monsoon	Shannon	Simpson	Chao1	Pre-monsoon	Shannon	Simpson	Chao1
Flooded	8.76	0.859	1733.2	Flooded	8.81	0.846	1866.48	Flooded	8.69	0.911	1908.32
Dry-wet	8.97	0.882	1809.5	Dry-wet	8.91	0.883	1903.75	Dry-wet	8.95	0.946	2044.65
Monsoon	Shannon	Simpson	Chao1	Monsoon	Shannon	Simpson	Chao1	Monsoon	Shannon	Simpson	Chao1
Flooded	8.29	0.841	1869.3	Flooded	8.77	0.861	1832.65	Flooded	8.56	0.871	1907.66
Dry-wet	8.43	0.867	1921.6	Dry-wet	8.49	0.841	1839.44	Dry-wet	8.77	0.884	1921.46
Gangetic Division 4 (Bihar)				Gangetic Division 5 (West Bengal)				Gangetic Division 6 (West Bengal)			
Field samples	Alpha diversity indices			Field samples	Alpha diversity indices			Field samples	Alpha diversity indices		
Pre-monsoon	Shannon	Simpson	Chao1	Pre-monsoon	Shannon	Simpson	Chao1	Pre-monsoon	Shannon	Simpson	Chao1
Flooded	9.13	0.911	2013.7	Flooded	9.25	0.921	2088.26	Flooded	9.37	0.923	2163.58
Dry-wet	9.62	0.949	2347.6	Dry-wet	9.44	0.953	2609.74	Dry-wet	9.71	0.9618	2691.35
Monsoon	Shannon	Simpson	Chao1	Monsoon	Shannon	Simpson	Chao1	Monsoon	Shannon	Simpson	Chao1
Flooded	8.41	0.807	1962.4	Flooded	8.52	0.869	1967.14	Flooded	8.62	0.859	1987.55
Dry-wet	8.92	0.883	2044.7	Dry-wet	8.73	0.899	2119.58	Dry-wet	8.79	0.896	2207.54

Note: Standard deviations were within 0.03-0.05 margin for the Shannon index, 0.007-0.009 margin for the Simpson index and 24.98-46.77 margin for the Chao1 index.

Supplementary Table S4. The volume of water (in L) which can be saved in AWD practice compared to the flooded.

Water-saving data related to the AWD practice								
Study divisions	Study sites	Pre-monsoonal DW	Pre-monsoonal FL	Monsoonal DW	Monsoonal FL	Average water consumption (DW)	Average water consumption (FL)	Per hectare water save through AWD
Upper Ganga	Site 1	24800	111600	16550	99100	20675	105350	1058438
	Site 2	25250	113400	17000	100900	21125	107150	1075313
	Site 3	25900	116550	17650	104050	21775	110300	1106563
	Site 4	23800	107100	15550	94600	19675	100850	1014688
Upper Middle Ganga	Site 5	27350	122850	19100	110350	23225	116600	1167188
	Site 6	27450	123300	19200	110800	23325	117050	1171563
	Site 7	28100	126450	19850	114950	23975	120700	1209063
	Site 8	29600	133200	21350	121700	25475	127450	1274688
Middle Ganga	Site 9	28750	129150	20800	117650	24775	123400	1232813
	Site 10	29500	132750	21550	121250	25525	127000	1268438
	Site 11	31400	141300	23450	129800	27425	135550	1351563
	Site 12	31650	142200	23700	124700	27675	133450	1322188
Middle Ganga	Site 13	32700	147150	24750	129650	28725	138400	1370938
	Site 14	32900	148050	24950	130550	28925	139300	1379688
	Site 15	36400	163800	28450	144300	32425	154050	1520313
	Site 16	33150	148950	25200	131450	29175	140200	1387813
Middle Lower Ganga	Site 17	34500	120750	24350	109250	29425	115000	1069688
	Site 18	36200	126700	26050	115200	31125	120950	1122813
	Site 19	35100	122850	24950	111350	30025	117100	1088438
	Site 20	31850	111300	21700	102800	26775	107050	1003438
Lower Ganga	Site 21	36750	128450	26600	119950	31675	124200	1156563
	Site 22	35900	125650	25750	117150	30825	121400	1132188
	Site 23	34350	120050	24200	111550	29275	115800	1081563
	Site 24	38600	135100	28450	122600	33525	128850	1191563

Note: Data are mean values with a SD of 1.24-2.08% range of the mean.

Supplementary Table S5. Profit calculation based on the rice productivity per hectare (H) in dry-wet (DW) and flooded (FL) fields. Average data for India has been collected from the Ministry of Agriculture database. Yields are in kg, profits are in INR.

Cost-benefit calculations and profit gained from yield data								
Study divisions	Study sites	Average Yield (DW)	Average Yield (FL)	Average yield India	Difference in Yield per H	Average Price yield India	Difference in price Yield per H	Average price DW
Upper Ganga	Site 1	5126.5	4899.5	2809	2317.5	57584.5	47508.75	105093.3
	Site 2	5225	4888.5	2809	2416	57584.5	49528	107112.5
	Site 3	5346	5047	2809	2537	57584.5	52008.5	109593
	Site 4	5616.5	5006.5	2809	2807.5	57584.5	57553.75	115138.3
Upper Middle Ganga	Site 5	5747	5155.5	2809	2938	57584.5	60229	117813.5
	Site 6	5776	4945	2809	2967	57584.5	60823.5	118408
	Site 7	5371.5	5144.5	2809	2562.5	57584.5	52531.25	110115.8
	Site 8	5526	5189.5	2809	2717	57584.5	55698.5	113283
Middle Ganga	Site 9	5523	5224	2809	2714	57584.5	55637	113221.5
	Site 10	5825.5	5215.5	2809	3016.5	57584.5	61838.25	119422.8
	Site 11	5920	5328.5	2809	3111	57584.5	63775.5	121360
	Site 12	6087	5256	2809	3278	57584.5	67199	124783.5
Middle Ganga	Site 13	5616.5	5389.5	2809	2807.5	57584.5	57553.75	115138.3
	Site 14	5827	5490.5	2809	3018	57584.5	61869	119453.5
	Site 15	5700	5401	2809	2891	57584.5	59265.5	116850
	Site 16	6034.5	5424.5	2809	3225.5	57584.5	66122.75	123707.3
Middle Lower Ganga	Site 17	6093	5501.5	2809	3284	57584.5	67322	124906.5
	Site 18	6398	5567	2809	3589	57584.5	73574.5	131159
	Site 19	5881	5058	2809	3072	57584.5	62976	120560.5
	Site 20	6073.5	5328	2809	3264.5	57584.5	66922.25	124506.8
Lower Ganga	Site 21	5865.5	5368	2809	3056.5	57584.5	62658.25	120242.8
	Site 22	5929	5387	2809	3120	57584.5	63960	121544.5
	Site 23	5946	5271	2809	3137	57584.5	64308.5	121893
	Site 24	6122.5	5378	2809	3313.5	57584.5	67926.75	125511.3

Note: Data are mean values with a SD of 1.62-2.11% range of the mean.

Supplementary Table S6. Profit calculations (in INR) based on the average income per hectare from dry-wet and flooded fields.

Cost-benefit calculations and average profit from monitory data							
Study divisions	Study sites	Pre-monsoonal DW	Pre-monsoonal FL	Monsoonal DW	Monsoonal FL	Average Profit (DW)	Average Profit (FL)
Upper Ganga	Site 1	67500.28	47347.43	58511.5	49892.33	63005.89	48619.88
	Site 2	68918.9	47766.86	59525.75	49210.86	64222.32	48488.86
	Site 3	70530.96	49272.98	60889.73	50852.6	65710.35	50062.79
	Site 4	76373.07	54852.74	61915.64	49613.55	69144.36	52233.14
Upper Middle Ganga	Site 5	92611.54	56316.74	72073.27	51255.29	82342.4	53786.01
	Site 6	88380.93	57938.73	76744.3	49707.18	82562.62	53822.96
	Site 7	81617.92	54894.87	71895.71	57499.06	76756.82	56196.96
	Site 8	84090.74	55948.11	73848.81	57385.8	78969.77	56666.96
Middle Ganga	Site 9	84105.64	66998.21	73753.2	57782.19	78929.42	62390.2
	Site 10	91330.44	67888.01	69872.16	56785.56	80601.3	62336.78
	Site 11	95188.64	69178.85	68986.1	58178.58	82087.37	63678.71
	Site 12	93013.75	72838.31	75061.94	57929.38	84037.84	65383.84
Middle Ganga	Site 13	102439.6	68389.31	69732.92	65595.78	86086.24	66992.54
	Site 14	106412.6	70344.36	83667.86	66162.75	95040.23	68253.55
	Site 15	104211.3	64913.14	81747.67	65065.79	92979.49	64989.47
	Site 16	113463.8	66123.73	83975.68	64375.56	98719.75	65249.65
Middle Lower Ganga	Site 17	117454.7	66911.2	82421.93	65447.87	99938.33	66179.53
	Site 18	117311.6	71965.09	91480.58	66773.58	104396.1	69369.33
	Site 19	118105.6	61246.13	79571.51	65361.09	98838.57	63303.61
	Site 20	123418	74354.73	81183.15	62935.86	102300.6	68645.3
Lower Ganga	Site 21	121706.9	71998.64	76676.01	66507.07	99191.45	69252.85
	Site 22	128053.9	71422.15	74053.68	67626.41	101053.8	69524.28
	Site 23	125328.1	71447.21	76389.19	64508.26	100858.6	67977.74
	Site 24	129585.9	73139.09	78287.66	65560.97	103936.8	69350.03

Note: Data are mean values with a SD of 1.49-2.26% range of the mean.