Microbial communities in paddy soils: differences in abundance and functionality between rhizosphere and pore water, the influence of different soil organic carbon, sulfate fertilization and cultivation time, and contribution to arsenic mobility and speciation

Microbial communities in paddy soils: Differences in abundance and functionality between rhizosphere and pore water, influence of different soil organic carbon, sulfate fertilization, and cultivation time, and contribution to arsenic mobility and speciation

Sarah Zecchin[a], Jiajia Wang[b], Maria Martin[c], Marco Romani[d], Britta Planer-Friedrich[b], Lucia Cavalca[a]*

[a] *Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano, Milano, Italy*

[b] *Environmental Geochemistry Group, Bayreuth Center for Ecology and Environmental Research (BAYCEER), Bayreuth University, Germany*

[c] *Department of Agriculture, Forest and Food Science, University of Turin, Turin, Italy*

[d] *Rice Research Centre, Ente Nazionale Risi, Castello d'Agogna, Pavia, Italy*

*Corresponding author. Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano, Milano, Italy.

E-mail address: lucia.cavalca@unimi.it

Abstract

Abiotic factors and rhizosphere microbial populations influence arsenic accumulation in rice grains.

Despite mineral and organic surfaces are keystones in element cycling, localization of specific microbial reactions in the root/soil/pore water system is still unclear. Here, we tested if original unplanted soil, rhizosphere soil, and pore water represented distinct ecological microniches for arsenic-, sulfur- and iron-cycling microorganisms and compared the influence of relevant factors such as soil type, sulfate fertilization, and cultivation time. In rice open-air-mesocosms with two paddy soils (2.0% and 4.7% organic carbon), Illumina 16S rRNA gene sequencing demonstrated little significant effects of cultivation time and sulfate fertilization that decreased *Archaea*-driven microbial networks and incremented sulfate reducing and sulfur oxidizing bacteria. Different compartments, characterized by different bacterial and archaeal compositions, had the strongest effect with higher microbial abundances, bacterial biodiversity and interconnections in the rhizosphere versus pore water. Within each compartment, a significant soil type effect was observed. Higher percentage contributions of rhizosphere dissimilatory arsenate- and iron-reducing, arsenite-oxidizing, and, surprisingly, dissimilatory sulfate-reducing bacteria as well as pore water iron-oxidizing bacteria in the lower organic carbon soil supported previous chemistry-based interpretations of a more active S-cycling, a higher percentage of thioarsenates, and lower arsenic mobility by sorption to mixed Fe(II)Fe(III)-minerals in this soil.

Keywords: rice paddy soil, rhizosphere microbiome, pore water microbiome, arsenic thiolation, sulfate fertilization

Introduction

Arsenic accumulates more in rice than in other crops, posing health concerns at a global level (Meharg et al., 2009). In aerobic soil environments, most of the metalloid is immobilized as arsenate in Fe(oxyhydr)oxides, while in flooded rice soils it is released mainly as arsenite by reductive dissolution.

Microorganisms methylate inorganic arsenic species to the less toxic mono- (MMA) and dimethylated (DMA) oxyarsenates which are also taken up by the plants from paddy soil porewater (Meharg and Zhao, 2012). Recently, thiolated arsenic forms have also been detected, both in paddy soil pore water (Wang et al., 2020b) and in rice grains (Colina Blanco et al., 2021). The reactions controlling the extent of arsenic dissolution and conversion into different chemical species depend on soil geochemical and physical factors and are often microbially mediated. Particularly, the plant rhizosphere in the paddy fields is characterized by steep gradients of redox conditions and physicochemical characteristics (pH, organic matter content, and redox-sensitive elements, such as arsenic, sulfur and iron) that shape microbial community even at microscale level.

Water management of the rice paddy was shown to strongly affect arsenic biogeochemistry by favoring specific microbial populations which can actively convert the different metalloid oxidation states. In rice field soil, continuous flooding promotes the presence of arsenic-solubilizing ferric iron- and arsenate-reducing bacteria (Zecchin et al., 2017a and b, 2019), while in aerobic rice field soil the predominance of ferrous iron- and arsenite-oxidizing bacteria leads to arsenic immobilization on the solid phase, lowering its concentrations in the pore water and in rice grains (Xu et al., 2008; Arao et al., 2009; Zecchin et al., 2017b; Li et al., 2019).

Besides water management, sulfate fertilization is a promising tool to decrease arsenic contamination in rice grain, acting both at the plant (*i.e.*, synthesis of phytochelatins) and at the soil level (Dahlawi et al., 2018; Zou et al., 2018; Chen et al, 2019 and 2021; Hu et al., 2007; Fang et al., 2023). The decreased concentration of arsenic in the pore water of sulfate-amended rice paddy soil was positively related to the presence of rhizospheric dissimilatory sulfate-reducing microorganisms (DSRM) (Jia et al., 2015) that, by producing sulfide in anoxic conditions at circumneutral pH, contribute to the removal of arsenic by secondary iron sulfides (Hu et al., 2007; Burton et al., 2014; Xu et al., 2019). Part of sulfide is used by sulfur-oxidizing bacteria (SOB), which contribute to the production of elemental sulfur (S^0)

71 in rice paddies (Stubner et al., 1998; Zhou et al., 2002; Friedrich et al., 2005; Hamilton et al., 2014).
72 Moreover, sulfide and S^0 are hypothesized to react abiotically with either arsenite or methylated
73 arsenates, to yield different inorganic and methylated thioarsenates (Planer-Friedrich et al., 2015; Fan
74 et al., 2018; Wang et al., 2020b).
75 Wang et al. (2020b) suggested that soil organic carbon (C) content plays an important role in the
76 biogeochemistry of arsenic by fueling microbial activity. In their study, the authors observed that
77 sulfate addition caused a stronger decrease of dissolved arsenic coupled to higher percentage of
78 methylation and thiolation in a low C soil, compared to a high C soil. The hypothesis was that in the
79 high C soil, reducing conditions lead to FeS mineral formation, a relatively large removal of reduced
80 sulfur from the pore water, and less active sulfur-cycling. In contrast, the lower C content caused less
81 pronounced reducing conditions (with higher Eh and less Fe(II) in the porewater), with consequently a
82 higher conversion of sulfide to S^0 and finally sulfate and increased adsorption of arsenic to mixed
83 valence iron minerals. The oxidized sulfur would then be available again for new organic C driven
84 reduction, promoting an active sulfur cycling.
85 The localization of arsenic, sulfur, and iron biogeochemical reaction sites in the soil/root/pore water
86 rice paddy system is an important but still overlooked aspect. In fact, it is not clear whether arsenic,
87 sulfur and iron transformations occur in solution or in the solid phases and which types of microbial
88 populations are crucial in regulating these reactions. Moreover, while the composition of the microbial
89 communities inhabiting different soil/plant compartments (*i.e.*, bulk soil, rhizosphere soil, rhizoplane,
90 endosphere) have been revealed by several authors (Somenahally et al., 2011; Zecchin et al., 2017a and
91 b; Das et al., 2016; Jia et al., 2014), to date, the microbial communities living in rice paddy pore water
92 have never been characterized, and their composition and role in element cycling is still unknown. A
93 previous study (Tian et al., 2021) suggested that in wetlands the water table level is positively related to
94 microbial species richness and diversity in the pore water. In light of recent issues with water scarcity,

which are driving the consideration of novel water-saving agronomic regimes, the ecological equilibrium of keystone arsenic, sulfur and iron-cycling microbial species in the pore water can be altered. In order to clarify if compartmentalization is a major driver of microbial communities involved in arsenic, sulfur and iron biogeochemistry in rice paddies, in the present study we characterized bacterial and archaeal populations inhabiting the original unplanted soil, the rhizosphere soil and the porewater of two rice paddy soils with different organic C content, non-fertilized and fertilized with sulfate, and tested whether the compartment effect leads to stronger differences in comparison with other factors such as sulfate fertilization, soil type (with low and high organic C), and cultivation time. Vice versa, the possible influence of the different microbial communities on the geochemical parameters was statistically evaluated to determine the role of specific microbial populations in arsenic, sulfur and iron cycling, focusing on total arsenic mobility and speciation, specifically thiolation and methylation.

Materials and methods

Experimental setup

The rice growing experiment was carried out at the Rice Research Center (Ente Nazionale Risi, ENR) in Castello d'Agogna (Pavia, Italy). The mesocosms were set up in the open air in 0.83 m^2 plastic tanks filled with 30 cm of soil from two distinct paddy fields located in Cascina Fornazzo and Cascina Veronica (Pavia, hereafter referred to as 'Fornazzo' and 'Veronica' soils, respectively). Fornazzo and Veronica soils were taken as representatives of high and low C soils, being characterized by 47 and 20 g kg^{-1} of organic C, respectively (Wang et al., 2020a). Arsenic concentrations were similar between the two soils with 5.6 and 5.8 mg kg^{-1}, respectively, which is below the Italian national limit for public use soil (20 mg kg^{-1}, D.Lgs. 152/2006). Furthermore, Fornazzo soil had slightly higher total S and Fe(II)

contents in comparison to Veronica soil (see Supplementary Table 1 for the complete characterization of the two soils). Absolute concentrations of dissolved total S and Fe(II) were lower in Veronica than in Fornazzo pore water (Supplementary Table 2) which reflected on the one hand side the differences in total S and Fe contents in the two soils (Supplementary Table 1). However, the proportion of Fe mobilized from soil to pore water was similar for both soils, while the proportion of S mobilized from Veronica soil was lower than Fornazzo soil (Supplementary Table 3), reflecting a higher overall redox potential in Veronica soil as described before (Wang et al., 2020b).

Rice plants (*Oryza sativa* var. Selenio) were water-seeded and cultivated under continuous flooding for the whole life cycle, using non-sterile tap water provided with a garden hose. Before seeding, mesocosms were fertilized with 100 kg ha^{-1} of either ammonium sulfate [$(NH_4)_2SO_4$] or urea (CH_4N_2O) as control nitrogen fertilizer. Further fertilization was applied at tillering stage with 30 kg ha^{-1} and at booting stage with 50 kg ha^{-1} of either urea or ammonium sulfate, according to the usual agronomic practices. For each type of fertilization (i.e., ammonium sulfate *vs* urea/control) and for each soil (i.e., Fornazzo *vs* Veronica), 3 replicates were set up. The physicochemical analyses were performed in the pore water over time by Wang et al. (2020a) and the results were summarized in Supplementary Tables 2 and 4.

Rhizosphere soil separation and DNA isolation

To analyze the microbial communities inhabiting the rice rhizospheric compartment, rhizosphere soil (*i.e.*, soil strictly attached to the roots), and pore water were collected during stem elongation, flowering and dough stage (corresponding to approximately 60, 80 and 100 days after seeding, respectively). These three rice life stages are considered crucial for both the development of rhizospheric microbial communities on expanding roots (Edwards et al., 2018) and for arsenic uptake, which is highest during flowering (Zheng et al., 2011). The original unplanted soil was sampled for the characterization of the

starting microbial community. For each experimental replicate, 3 plants were collected and pooled in one composite sample. Roots were shaken in tetrasodium pyrophosphate and the rhizosphere soil was separated from roots according to Zecchin et al. (2017b). Pore water was sampled with 15 µm-pore size Rhizon samplers (Rhizon SMS 5 cm, Rhizosphere, Wageningen, The Netherlands) and planktonic cells were collected on cellulose acetate filters (0.2 µm pores) with a vacuum pump. DNA was isolated from all samples using DNeasy PowerSoil kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The quality of the isolated DNA was checked under UV light by agarose gel electrophoresis on a 1% Tris-acetate-EDTA (TAE) agarose gel, stained with GelRed (Biotium, CA, USA).

Illumina 16S rRNA genes libraries

From DNA isolated from the original soils, rhizosphere soil and pore water samples, bacterial and archaeal 16S rRNA genes were sequenced with primers 341F/806R (5'- CCTACGGGAGGCAGCAG-3'/5'- GGACTACHVGGGTWTCTAAT-3') and 344F/806R (5'-CCCTAYGGGGYGCASCAG-3'), respectively (Rago et al., 2017). Sequencing was performed on 1 and 0.1 µg of DNA for rhizosphere soil and pore water, respectively, at the DNA Services (DNAS) facility, Research Resources Center (RRC), University of Illinois at Chicago (UIC, USA). Raw reads were processed and analyzed with QIIME2 (https://qiime2.org/, Bolyen et al., 2019). The DADA2 workflow (Callahan et al., 2016) was used to remove barcodes and sequence adapters, filter high quality non-chimeric reads, cluster the reads in single amplicon sequence variants (ASVs) and pick one representative sequence for each ASV. Alpha diversity was estimated upon rarefaction of the datasets. Microbial species richness was determined by calculating the number of observed microbial species and using the Chao1 richness estimator (Chao, 1984), while microbial species evenness was estimated according to Pielou's algorithm (Pielou, 1966). The taxonomy of representative sequences was assigned using the SILVA

SSU reference dataset version 138 (https://www.arb-silva.de/). The taxonomic classification was performed using a naïve Bayes classifier optimized for the primers used in the sequencing process (Bokulich et al., 2018; Pedregosa et al., 2011). ASV tables were obtained to determine the relative abundance of each taxon in the samples. Representative sequences were aligned with mafft (Katoh and Standley, 2013) and phylogenetic analysis of the representative sequences was performed with FastTree (Price et al., 2010).

Functional prediction

The presence in the library of microorganisms related to arsenic, sulfur and iron cycles and in methanogenesis [*i.e.*, dissimilatory arsenate reducing bacteria (DAsRB), arsenate-reducing bacteria (AsRB), arsenite-oxidizing bacteria (AsOB), arsenite-methylating bacteria (AsMB), dissimilatory sulfate-reducing bacteria (DSRB), sulfur-oxidizing bacteria (SOB), dissimilatory Fe(III)-reducing bacteria (DFeRB), Fe(II)-oxidizing bacteria (FeOB)] was evaluated according to a reference database of microbial genera retrieved according to the literature and to data available at the National Center for Biotechnology Information (NCBI, Supplementary Dataset 1). Methanogenic archaea (*i.e.*, MA) were retrieved using the PhyMET^2 database (http://phymet2.biotech.uni.wroc.pl/, Burdukiewicz et al., 2018). The R-based package Tax4Fun2 (Wemheuer et al., 2020) was used to infer the presence of genes related to arsenic, sulfur and iron metabolisms as well as to methanogenesis and methanotrophy.

Quantification of microorganisms involved in arsenic, sulfur and iron transformations by real-time qPCR

To further analyze microorganisms putatively involved in arsenic cycling in rice rhizosphere and in pore water, the 16S rRNA genes of total bacteria and archaea and genes encoding the A subunit of arsenite oxidase (*aioA*), arsenate reductase (*arsC*), the A subunit of dissimilatory arsenate reductase

191 (*arrA*), arsenite methyltransferase (*arsM*) and the A subunit of dissimilatory bisulfite reductase (*dsrA*)
192 were amplified and quantified by real time qPCR (RT-qPCR). Furthermore, 16S rRNA genes of the
193 microorganisms belonging to iron-reducing *Geobacteriaceae* and *Shewanellaceae* and to iron-
194 oxidizing *Gallionellaceae* were quantified. Details of primer pairs and protocols used in this study can
195 be found in Supplementary Table 5. For each reaction, 10 ng of template DNA were mixed with
196 primers and Titan HotTaq EvaGeen® qPCR Mix (Bioatlas, Estonia), in a total volume of 20 µL. The
197 thermal protocols were carried out on a QuantStudio™ 3 System (Thermofisher, Waltham,
198 Massachusetts, USA). The correct size of qPCR amplicons was checked by agarose gel electrophoresis.
199 Standard curves were created by the amplification of the selected target from plasmid DNA
200 (Supplementary Table 5). The abundance of the quantified functional genes was expressed as relative
201 abundance by normalization to total bacterial and archaeal 16S rRNA genes, while the 16S rRNA
202 genes of iron cycling bacteria were normalized only to total bacterial 16S rRNA genes.
203
204 **Statistical analysis**
205 The statistical analyses of Illumina 16S rRNA gene library data were performed using QIIME2 and the
206 R program, v.3.6.0 (R Core Team, 2015), package vegan version 2.5-5 (Oksanen et al., 2020).
207 With the R base program, one-way analysis of variance (ANOVA), Tukey's b, Duncan and t-test at $p \leq$
208 0.05 were used for comparisons in the analysis of the alpha diversity, of the abundance of
209 microorganisms related to arsenic, sulfur and iron cycles and of the qPCR amplifications. The alpha
210 diversity was analyzed by gathering the samples in different groups to evaluate the "compartment
211 effect" (*i.e.*, original unplanted soil *vs* rhizosphere soil *vs* pore water), the "soil type effect" (*i.e.*,
212 Fornazzo *vs* Veronica), the "sulfate amendment effect" (*i.e.*, control *vs* sulfate), and the "time effect"
213 (*i.e.*, stem elongation *vs* flowering *vs* dough).
214 To compare bacterial and archaeal diversity among the samples, weighted UniFrac distances were

calculated from rarefied ASV tables and principal coordinates analysis (PCoA) was performed (Lozupone et al., 2005; Hamady and Knight 2009; Halko et al., 2010). Significantly different groups of samples defined by the "compartment effect", the "soil type effect", the "sulfate amendment effect", and the "time effect" were identified applying the permutational analysis of variance (PERMANOVA, permutations = 999), using the QIIME2 pipeline (Anderson, 2001).

Significant differences in the abundance (*i.e.*, differential abundance) of bacterial and archaeal families and genera retrieved with 16S rRNA genes Illumina sequencing due to the different soils and to sulfate application were tested using the quasi-likelihood F-test implemented in the R package EdgeR version 3.11 (Robinson et al., 2010; R Core Team, 2015).

To highlight statistically significant positive and negative interactions among bacterial and archaeal genera, co-occurrence network analysis was performed by testing the probabilistic co-occurrence model on presence-absence genus tables using the R package cooccur version 1.3 (Veech, 2013; Griffith et al., 2016). Positive and negative correlations were tested by grouping original unplanted soil, rhizosphere soil and pore water samples collected according to the compartment (*i.e.*, original unplanted soil *vs* rhizosphere soil *vs* pore water), soil type (*i.e.*, Fornazzo *vs* Veronica), sulfate amendment (*i.e.*, control *vs* sulfate) and timing (*i.e.*, stem elongation *vs* flowering *vs* dough). Co-occurrence analysis is based on presence/absence of each genus in the samples. The genera that were not present in all the replicates of at least one sample with at least 20 reads were removed from the analysis. To estimate the number of possible keystone genera, each network was re-calculated by removing one genus and calculating the percentage of lost connections without that genus. This process was repeated for all genera.

To investigate links between chemistry and microbial populations involved in arsenic, sulfur and iron cycles, linear Pearson correlations were calculated between the relative abundance of the different microbial populations in the rhizosphere soil and in the pore water and pore water physicochemical

parameters (*i.e.*, total arsenic, ferrous iron, total sulfur, methylated arsenic, methylated oxyarsenates, total thioarsenates, inorganic thioarsenates, methylated thioarsenates, total organic carbon (TOC), total inorganic carbon (TIC), pH and Eh) at each time point.

Possible statistically significant correlations between the bacterial and archaeal community compositions, the functional predictions, the physicochemical parameters measured in the pore water (*i.e.* total arsenic, ferrous iron, total sulfur, methylated arsenic, methylated oxyarsenates, total thioarsenates, inorganic thioarsenates, methylated thioarsenates, total organic carbon (TOC), total inorganic carbon (TIC), pH and Eh) and qPCR data were evaluated applying the redundancy analysis (RDA, Legendre and Legendre, 2012) and the Mantel test (permutations = 999), both implemented in the vegan package (Legendre and Legendre, 2012). Bacterial and archaeal genera abundance data, the relative abundance of microorganisms involved in arsenic, sulfur and iron cycles (as indicated in Supplementary Dataset 1), and the relative abundance of enzymes involved in arsenic, sulfur and iron cycles (as indicated in Supplementary Datasets 2 and 3) were Hellinger-transformed to calculate Bray-Curtis dissimilarities, while the physicochemical and qPCR data were log-transformed to calculate Euclidean dissimilarities (Legendre and Gallager, 2001).

Data availability

The raw reads obtained with Illumina sequencing of 16S rRNA genes were deposited in GenBank within the Bioproject PRJNA858795 and in the Dataverse repository (https://dataverse.unimi.it/dataverse/P-RICE).

Results

Diversity of rice rhizosphere bacterial and archaeal communities

Illumina sequencing of 16S rRNA genes produced in total 330,206 and 551,058 high quality bacterial and archaeal reads, respectively (Supplementary Table 6). On average, the rhizosphere soil showed a higher number of ASVs than the pore water. This difference was more pronounced in the bacterial *vs* archaeal library and more pronounced in the higher organic C soil Fornazzo *vs* the lower organic C soil Veronica (Supplementary Table 6). Accordingly, bacterial and archaeal 16S rRNA genes biomarkers were higher in the higher organic C soil in both rhizosphere soil and pore water (data not shown). Both bacterial species richness (Chao1 index) and evenness were significantly lower in the pore water with respect to the original unplanted soil, while an opposite trend was observed for *Archaea*, which were significantly richer and more uniform in the pore water samples compared to the original unplanted soil and to the rhizosphere soil (Figure 1A, $p \leq 0.05$). A soil type effect was observed for both Bacteria and Archaea in all compartments, each following different patterns (Supplementary Figure 1A, $p \leq 0.05$). Archaeal Chao1 index negatively responded to sulfate amendment, being lower in all rhizosphere soil and pore water samples where sulfate was supplied, compared to the controls (Supplementary Figure 1B, $p \leq 0.001$). In the rhizosphere soil, both bacterial and archaeal Chao1 index significantly decreased, while in the pore water the trend was more variable (Supplementary Figure 1C, $p \leq 0.05$).

PCoA analysis based on Weighted UniFrac revealed a significant "compartment effect" in both bacterial and archaeal communities ($p \leq 0.01$, Figure 1B). When analyzing the beta diversity dividing soil (*i.e.*, original unplanted soil and rhizosphere soil) and pore water samples, a significant "soil type effect" was observed in both bacterial and archaeal communities in all compartments, while sulfate amendment and time effects were significant only in soil samples ($p \leq 0.05$, Supplementary Figure 2A and B).

Composition of rice rhizosphere bacterial and archaeal communities

Soil and pore water samples showed highly different composition in both bacterial and archaeal communities, evidencing a strong compartment effect (Figure 2). In soil samples, the predominant bacterial phyla were *Proteobacteria*, *Actinobacteriota* (former *Actinobacteria*)*, Firmicutes*, *Acidobacteriota* (former *Acidobacteria*), and other uncharacterized *Bacteria* (relative abundance 20-30%; Figure 2A). In the pore water, uncharacterized *Bacteria* (relative abundance 40-60%), *Proteobacteria* and *Patescibacteria* were the most abundant, and sulfate amendment increased the relative abundance of *Epsylonproteobacteraeota* (former class *Epsilonproteobacteria*) with the concomitant decrease of *Patescibacteria*. The compartment effect was evident also within *Proteobacteria*, being more abundant in soil samples, with the exception of *Gammaproteobacteria* that had their highest abundance in the pore water of Veronica soil (Supplementary Figure 3).

Concerning the archaeal communities, soil samples were dominated by *Euryarchaeota* (> 70%, including *Methanomicrobia*, and *Methanobacteria*), followed by *Crenarchaeota* (*i.e.*, *Bathyarchaeia*) and *Thaumarchaeota* (*i.e.*, *Nitrososphaeria*, Figure 2B). In the pore water, uncharacterized archaeal phyla were dominant (relative abundance 50-70%), followed by *Methanomicrobia*, *Woesearchaeia* and *Nitrososphaeria*.

Differential abundance analysis was performed at the genus level in all compartments at the flowering stage to evaluate the "soil type effect" and the "sulfate amendment effect". In soil and pore water samples, different genera were significantly affected by the soil type and sulfate fertilization (Figure 3). Most of the genera (*i.e.*, 35 genera belonging to the *Acidobacteriota*, *Actinobacteriota*, *Bacteroidota* (former *Bacteroidetes*), *Cyanobacteria*, *Firmicutes*, *Nitrospirota* (former *Nitrospirae*), *Alphaproteobacteria*, *Gammaproteobacteria*, *Methanomicrobia*, *Thermoplasmata* and *Nitrososphaeria*) were significantly driven by soil type rather than by sulfate fertilization (Figure 3, $p \leq 0.05$). Sulfate fertilization significantly decreased the abundance of uncharacterized *Elsterales* in

310 Veronica and of *Methanoregula* in Fornazzo in the rhizosphere soil, while in the pore water
311 uncharacterized *Campylobacterales* and *Burkholderiaceae*, *Ferritrophicum*, *Methylomonas*,
312 *Methanobacterium* and *Candidatus* Nitrosotalea significantly increased in sulfate-amended samples (p
313 ≤ 0.05).
314 Co-occurrence network analysis (Figure 4) revealed that the compartment, the soil type, sulfate
315 amendment and timing significantly affected specific correlations between microbial genera. In fact, 1)
316 the number of connections was higher in unplanted original soil and in rhizosphere soil than in pore
317 water (*i.e.*, "compartment effect"), 2) a higher number of nodes and connections were present in lower
318 C Veronica soil than in higher C Fornazzo soil (*i.e.*, "soil type effect"), 3) less connections were
319 observed in sulfate-amended samples (*i.e.*, "sulfate amendment effect"), and 4) the number of
320 connections increased over time (*i.e.*, "time effect") (Figure 4A, Supplementary Dataset 4).
321 To evaluate the presence of possible keystone genera, data concerning the number of connections that
322 were lost when each genus was removed (results shown in Supplementary Dataset 5) were compared
323 with the number of genera that show a high number of connections (Supplementary Figure 4), with the
324 proportion of genera responsible for the loss of at least one connection and the percentage of the
325 maximum number of lost connections (Supplementary Figure 5). The networks based on the
326 rhizosphere soil, the pore water and the stem elongation showed the lowest proportion of genera
327 responsible for the loss of at least one connection (Supplementary Figure 5), suggesting the presence of
328 a lower number of potential keystone genera in these samples if compared with the others. However, in
329 the networks with the highest number of nodes and connections (*i.e.*, Fornazzo, Veronica, control,
330 sulfate, flowering and dough) the maximum number of lost connections from genera removal is lower
331 with respect to the other networks. This might indicate that the trophic networks and the ecological
332 niches in the samples characterized by the same soil type and by the same fertilization type and the
333 ones established at the flowering and dough stages are likely more stable, probably because the same

functions can be completed by different microbial genera. Therefore, the loss of one genus does not compromise the presence of a specific microbial function in the ecosystem, due to functional redundancy.

The highest number of positive and negative connections was observed between *Alphaproteobacteria*, *Patescibacteria* and *Actinobacteriota*, followed by *Thaumarchaeota*, *Acidobacteriota*, *Bacteroidota* and *Chloroflexi* (Figure 4B). The genera with the highest number of positive connections were mostly affiliated to archaeal phyla, such as *Thaumarchaeota*, *Euryarchaeota* and *Crenarchaeota*, together with uncharacterized members of the *Acidobacteriota* family *Blastocatellaceae* (Supplementary Dataset 6). *Nitrospirota* showed the highest number of connections in proportion to the number of genera present in the phylum (Supplementary Figure 6), explained by the presence of only one uncharacterized genus within the order *Thermodesulfovibrionia*, significantly positively related to *Proteobacteria* genera (*i.e.*, *Rhizobiales* genera, *Myxococcales* genera, *Sphingomonas*, *Comamonas*, *Desulfobacterium* and *Acinetobacter*).

In the sulfate-amended samples, a lower number of connections was mostly ascribable to a lower number of connections related to all archaeal phyla, concomitant to a higher number of connections of the genera *Desulfobacterium*, *Comamonas* and *Pseudomonas* when sulfate was applied (Supplementary Dataset 6).

Inferred microbial functionalities and biomarkers related to arsenic, sulfur and iron biogeochemical cycles

Microbial functionalities related to arsenic, sulfur and iron cycles in the analyzed samples were inferred on the basis of the genera retrieved with Illumina sequencing of 16S rRNA genes (Supplementary Dataset 1).

All the retrieved genera involved in arsenic, sulfur and iron cycles were in general more abundant in

the rhizosphere soil than in the pore water (Supplementary Figure 7A), indicating that microbial populations of this compartment contribute mostly to those elemental cycling. These outcomes suggest that the compartment was the strongest driver if compared to soil type, sulfate amendment and timing. A significant effect was exerted by the soil type on DAsRB/DFeRB and on AsOB in the rhizosphere soil, and on pore water SOB and FeOB (Figure 5). The two versatile genera *Bacillus* and *Geothermobacter* able to perform dissimilatory respiration of both arsenate and ferric iron were the only contributors to the group DAsRB/DFeRB (Supplementary Dataset 1).

Within sulfur cycling, a higher number of SOB was revealed (*i.e.*, *Bacillus*, *Acidiphilium*, *Azospirillum*, *Methylobacterium*, *Bradyrhizobium*, *Rhodopseudomonas*, *Paracoccus*, *Acidithiobacillus*, *Comamonas* and *Polaromonas*) with respect to DSRB (Supplementary Figure 6, Supplementary Dataset 1). A "sulfate amendment effect" was observed only for DSRB (*i.e.*, unclassified *Thermodesulfovibronia*, *Desulfobacterium*, *Desulfovibrio* and unclassified *Desulfobulbaceae*), which were generally within the "rare biosphere" (i.e., relative abundance < 1%), although significantly more abundant in sulfate-amended rhizosphere soil samples (Figure 5).

Some of the bacterial and archaeal genera that were involved in arsenic, sulfur and iron and methane cycles (*i.e.*, included in Supplementary Dataset 1) were also involved in positive and/or negative correlations according to the co-occurrence analysis. These genera are highlighted in Supplementary Dataset 6. Specifically, MA belonging to the genera *Methanomassiliicoccus* and *Methanoculleus* showed more than 100 positive connections with other genera (Supplementary Dataset 6). A number of directly and indirectly arsenic-cycling bacterial genera showed a high number of positive connections, with *Clostridium*, *Mesorhizobium*, *Bradyrhizobium*, uncharacterized *Thermodesulfovibrionia*, *Desulfobacterium* and *Comamonas* among the most connected ones (Supplementary Dataset 6).

To implement the information on microbial functions inferred by the presence of specific microbial genera in the 16S rRNA gene library, predicted enzymes were investigated by Tax4Fun2 and specific

382 gene biomarkers were quantified by Real Time qPCR at the flowering stage.

383 The ubiquitous arsenate detoxification system ARS (*i.e.*, arsenate reductase ArsC, arsenite efflux pump
384 ArsB) was detected in the pore water and in rhizosphere soil, where *arsC* was in the order of 10^4 and
385 10^8 copies per g of mL/dry soil, respectively, reflecting the ability of the Arsenate reductase to use
386 soluble arsenic (Figure 6, Supplementary Figure 7B). Arsenite oxidase coded by *aioA* gene was present
387 only in rhizosphere soil at 10^8 copies per g of dry soil, together with arsenite methyltransferase *arsM*
388 which was retrieved also in the pore water of higher organic C soil Fornazzo (Figure 6, Supplementary
389 Figure 7B). Here, the higher C content might have favored the presence of methylated groups. Genes
390 encoding the respiratory arsenate reductase (i.e., ArrA) and the anaerobic arsenite oxidase (i.e., ArxA)
391 were not retrieved by Tax4Fun2, nor by Real Time qPCR, according to previous evidence in rice paddy
392 soil from the same area (Zecchin et al., 2017a).

393 Sulfur cycle related enzymes showed a compartment-dependent pattern, with sulfur oxidase (SoxAB)
394 and thiosulfate reductase more abundant in rhizosphere soil samples, and enzymes involved in
395 dissimilatory sulfate respiration (DsrAB) and respective biomarker *dsr* being absent in the pore water
396 (Figure 6, Supplementary Figure 7B). Sulfate amendment increased the abundance of enzymes
397 involved in dissimilatory sulfate respiration (DsrAB) in the rhizosphere sample of the lower carbon soil
398 Veronica, and SoxAB in the pore water (Figure 6, Supplementary Figure 7B).

399 A "compartment effect" was observed for the relative abundance of most targets, with *aioA* and *dsr*
400 only detected in rhizosphere soil samples, and *arsC* and *arsM* more abundant in the rhizosphere soil
401 compared to the pore water (Figure 6)

402 Regarding the iron cycle, DFeRB of the families *Geobacteraceae* and *Shewanellaceae* were more
403 abundant in the pore water compared to the rhizosphere soil (Figure 6), with *Shewanellaceae*
404 significantly more abundant in Veronica samples ($p \leq 0.05$). Sulfate amendment significantly increased
405 the abundance of *Gallionellaceae* in Veronica rhizosphere soil, and of *Geobacteraceae* in both

406 Fornazzo and Veronica, compared to the controls ($p \leq 0.05$).

407 In general, microbial functions were more represented in the rhizosphere compartment with respect to
408 the soil pore water. This habit reflects either the higher bacterial biodiversity that characterizes the soil
409 compartment, and the fact that a large part of the pore water microbial community comprised
410 uncharacterized ASVs. For such ASVs the prediction of functions might fail to give a correct picture.

411

412 **Correlation among microbial diversity, functionality, and environmental parameters**

413 The correlation among the microbial community composition and functionality in the samples and the
414 main physicochemical parameters measured in the porewater (*i.e.*, total arsenic, total sulfur, ferrous
415 iron, total thioarsenates, inorganic thioarsenates, methylated thioarsenates, methylated oxyarsenates,
416 TOC, TIC, pH and Eh) as well as the qPCR quantifications were investigated by RDA analysis (Figure
417 7) and Mantel test (Supplementary Tables 7, 8 and 9).

418 In both the rhizosphere soil and the porewater, the beta diversity of bacterial and archaeal communities
419 was significantly driven by TIC, ferrous iron and total sulfur concentrations (all higher in Fornazzo
420 samples; $p \leq 0.05$, Figure 7A, Supplementary Table 7). TOC significantly shaped the bacterial and
421 archaeal communities in the rhizosphere soil ($p \leq 0.05$, Figure 7A, Supplementary Table 7). pH was
422 significantly correlated with the bacterial communities in both the rhizosphere soil and the pore water
423 ($p \leq 0.05$, Figure 7A, Supplementary Table 7). In the rhizosphere soil, the bacterial community was
424 significantly shaped by total arsenic concentration ($p \leq 0.05$, Figure 7A, Supplementary Table 7). On
425 the other hand, the archaeal community living in the pore water was significantly related to the
426 concentration of methylated thioarsenates ($p \leq 0.05$, Figure 7A, Supplementary Table 7).

427 *Shewanellaceae* 16S rRNA and *arsM* genes quantified by qPCR were significantly related to the beta
428 diversity of both bacterial and archaeal communities in both the rhizosphere soil and pore water

429 samples, being more abundant in Veronica samples ($p \leq 0.05$, Figure 7A, Supplementary Table 7). In
430 the pore water, both bacterial and archaeal communities were significantly related to *Gallionellaceae*
431 16S gene copy number that was higher in Veronica samples ($p \leq 0.05$, Figure 7A, Supplementary Table
432 7).
433 The RDA of the functionalities inferred in the samples from Illumina sequencing (*i.e.*, presence of
434 specific genera *vs* Tax4Fun2) showed a similar pattern in the rhizosphere soil, were the abundance of
435 microbial species (*i.e.*, DAsRB/DFeRB, AsRB, AsOB, AsMB, FeOB and SOB) and enzymes (ArsC,
436 ArsB, ArsM, ArsH, DsrAB and Sox) involved in arsenic, sulfur and iron cycles were shaped by TIC,
437 TOC, total sulfur, and ferrous iron and were related to *arsM* gene copy number ($p \leq 0.05$, Figure 7B
438 and 7C, Supplementary Tables 8 and 9). On the other hand, the distribution of the specific genera in the
439 pore water samples was shaped by total sulfur and significantly related to *arsC* and *arsM* gene copies
440 ($p \leq 0.05$, Figure 7B, Supplementary Table 8), while Tax4Fun2-inferred enzymes were significantly
441 related to total sulfur and ferrous iron, as well as to the concentration of methylated arsenic ($p \leq 0.05$,
442 Figure 7C, Supplementary Table 9).
443 To further investigate whether the dynamics in the microbial populations observed in this study support
444 previously reported differences in chemistry between the two soils, Pearson linear correlation tests
445 were performed between different pore water chemistry parameters (*i.e.*, total arsenic, total sulfur, total
446 thioarsenates, inorganic thioarsenates, methylated thioarsenates, methylated oxyarsenates, total organic
447 carbon (TOC), total inorganic carbon (TIC), pH and Eh) and rhizospheric and pore water arsenic, sulfur
448 and iron cycling microbial populations.
449 While As did not show any correlation with microbial populations involved in arsenic, sulfur and iron
450 cycles, Fe(II), total S, TIC and TOC were significantly correlated with different microbial populations,
451 showing different trends (Supplementary Table 10). Specifically, Fe(II) was negatively correlated with
452 rhizospheric AsOB and DSRB, while total S, TIC and TOC were negatively correlated with

453 rhizospheric DAsRB/DFeRB, AsOB, DSRB but positively correlated to rhizospheric FeOB.
454 Interestingly, rhizospheric SOB were positively correlated with different thiolated and methylated As
455 species (i.e., total thioarsenates, inorganic thioarsenates, methylated thioarsenates). Pore water FeOB
456 were negatively correlated with Fe(II) and total S, but positively correlated with methylated arsenic.
457 Rhizospheric DAsRB/DFeRB, AsOB and DSRB, and pore water FeOB are significantly negatively
458 driven by Fe(II) and total S, which are lower in Veronica soil. This outcome is in accordance with
459 previously shown data (in Figure 5), where these populations were more abundant in Veronica soil.
460 Moreover, these data suggest a link between SOB and arsenic thiolation and between FeOB and
461 methylated arsenic.
462

463 ## Discussion

464 To the best of our knowledge, the analyses performed in the present study revealed for the first time
465 that paddy field pore water harbors specific microbial populations that are distinct from the ones
466 inhabiting the original unplanted soil and the rhizosphere soil, thus confirming that the compartment,
467 likely characterized by different physico-chemical properties, is the major driver in shaping these
468 different ecosystems. In fact, previous studies on rice paddy soil compartments were only focused on
469 unplanted, bulk or rhizosphere soil, and demonstrated the existence of a "rhizosphere effect" due to the
470 presence of a high amount of root exudates coupled to O_2 leaking from root aerenchyma that together
471 fuel microbial organic matter degradation, respiration, and fermentation processes (Lynch and Whipps,
472 1990; Revsbech et al., 1999; Liesack et al., 2000; Demoling et al., 2007; Marschner, 2011; Wörner et
473 al., 2016; Huaidong et al., 2017; Ding et al., 2019).
474 Unexpectedly, archaeal diversity was found to be higher in the pore water with respect to the original
475 unplanted soil and to the rhizosphere soil. While a sharp separation of the rhizosphere soil samples
476 from the bulk soil was ensured by the protocol followed (in accordance with Zecchin et al., 2017b), we

cannot ensure that the sampled pore water was derived exclusively from the rhizosphere area. Hence, in the pore water samples, microorganisms deriving from the anoxic bulk soil area were likely included. One hypothesis could be that a proportionally higher number of anaerobic and oligotrophic archaeal species are present in the pore water compartment, compared to the bacterial community. This possible explanation might be found in the lifestyle of pore water archaeal species, which is however still poorly inferable since most of the retrieved genera were uncharacterized. These outcomes suggest the importance of further investigating microbial communities in rice paddy pore water in order to clarify the role of uncharacterized microbial taxa in element cycling.

While the rhizosphere soil was dominated by *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, and methanogenic Archaea, typically found in rice rhizosphere (Ding et al., 2019; Bao et al., 2016), the pore water microbial communities were more related to aquatic ecosystems, and mostly hosted uncharacterized microbial species. Among these, members of the phylum *Patescibacteria,* including microbial taxa formerly assigned to the "Candidate Phyla Radiation" (CPR) group (Parks et al., 2018), were dominant. These microorganisms are widely distributed in aquatic subsurface environments, where they were suggested to have a fermentative lifestyle and to be associated to autotrophic iron- and sulfur-cycling microorganisms (Herrmann et al., 2019). Pore water archaeal community hosted a significantly higher proportion of members of the DPANN archaeal superphylum (*i.e.*, *Diapherotrites*, *Parvarchaeota*, *Aenigmarchaeota*, *Nanoarchaeota* and *Nanohaloarchaeota*) compared to the rhizosphere soil. This superphylum includes a variety of still poorly characterized small-sized microorganisms with diverse metabolic features, supposed to be widespread in the environment (Moissl-Eichinger et al., 2018). The presence of DPANN in agricultural soils, including rice paddies, was recently reported, however, without further discussion (Cho et al., 2022; Wan et al., 2021). Members of the phylum *Nanoarchaeota* include putative sulfide-oxidizers that live as obligate endosymbionts of *Crenarchaeota* (St. John et al., 2019), and might play a crucial role in sulfur cycling

in rice paddies and in plant detoxification from reduced sulfur compounds.

Interestingly, both Illumina sequencing and qPCR indicated that the relative abundance of most of the microorganisms involved in arsenic, sulfur and iron cycles were in general more abundant in the rhizosphere soil compared to the pore water, suggesting that organic matter, surface of soil particles and minerals have a crucial role in mediating microbial reactions in rice field soil, thus influencing the element biogeochemical cycles as reported before (Hoffman et al., 2021; Crundwell, 2013). However, since several uncharacterized bacterial and archaeal genera were retrieved in the pore water, the relative importance of pore water microbial communities with respect to rhizosphere soil in element cycling should be confirmed by further investigations.

Overall, the results underline that the "soil type" is defined by a complex of physico-chemical parameters (*i.e.*, mainly organic C content, iron and sulfur) that were the main drivers in the taxonomic and functional shaping of the microbial communities, rather than sulfate addition. In fact, within the strong influence determined by the compartment, the two soils were originally characterized by distinct microbial communities, with differentially abundant bacterial and archaeal genera, and over time the differentiation was maintained both in the composition and in the ecological networks despite a similar agronomic management. Moreover, the soil type strictly defined specific physicochemical parameters (organic and inorganic C, iron, sulfur, and pH) that selected specific microbial populations directly or indirectly involved in arsenic cycling. This confirms the relevance of pore water C content and redox potential in shaping the rhizosphere microbial populations involved in arsenic biogeochemistry in rice paddies, hypothesized in previous studies (Somenahally et al., 2011; Zecchin et al., 2017a; Yang et al., 2018 and 2020; Ma et al., 2014; Hossain et al., 2021; Dai et al., 2020). Microbial communities in the rhizosphere soil and in the pore water were differently influenced by pore water C. In fact, while pore water TIC and TOC were significantly related to both the phylogenetic and functional diversity of the rhizosphere microbial communities, these parameters were only weakly or not related to the pore water

525 microbiome. The presence of several uncharacterized microbial genera in the pore water might have
526 biased these outcomes. In this regard, the soil type had a certain but rather minor role. In fact, the lower
527 organic C content in Veronica soil corresponded to a lower microbial proliferation in comparison with
528 Fornazzo soil, as demonstrated in the present study by absolute qPCR quantification, but this difference
529 was not reflected by a lower number of microbial species. This might indicate that not only the
530 concentration but also the quality of organic C substrates inherited from the soil is important for
531 microbial community shaping. Some works suggest that the soluble C released from the added rice
532 straw is rapidly utilized, supplying easily degradable electron donors that may prime microbially-
533 catalyzed reductive dissolution of soil iron minerals; however, it is the progressive release of
534 previously iron-stabilized organic C that feeds the microbial communities during the whole growing
535 season (Said-Pullicino et al., 2016; Ye and Howrath, 2017).
536 The outcomes from the present study showed that sulfate amendment suppressed several positive and
537 negative correlations driven by archaeal genera, probably not only in relation to sulfate but to a general
538 higher nutrient availability in rice rhizosphere in comparison to the unamended mesocosms. This aspect
539 emerged also in the study of Liu et al. (2021), where alternating wet-dry cycles were found to be more
540 efficient than sulfate fertilization in decreasing CH_4 production in Veronica straw-amended soils. The
541 hypothesis is that in soils with high C content, sulfate fertilization might not be crucial for DSRB to
542 outcompete MA.
543 In the present study, sulfate amendment increased the relative abundance of DSRB, as previously
544 observed by Wörner et al. (2016), and SOB. Many genera that positively responded to sulfate
545 amendment were uncharacterized bacteria and archaea. For some of these, the presence of sulfur
546 cycling as a crucial metabolic trait was inferred by previous in vivo studies, as for the DSRB class
547 *Thermodesulfovibronia* (*i.e.*, phylum *Nitrospirota*; Sekiguchi et al., 2008; Umezawa et al., 2021;
548 Umezawa et al., 2020; Zecchin et al., 2018; Anantharaman et al., 2016 and 2018; Arshad et al., 2017;

549 Kato et al., 2018) and for the SOB *Campylobacterales* (Tan and Foght, 2014; Sievert et al., 2007;
550 Inagaki et al., 2003 and 2004; Kodama and Watanabe, 2004; Stolz et al., 2015). These observations,
551 coupled to a relatively high abundance of SOB, support previous hypotheses that sulfur cycling occurs
552 at high rates in rice rhizosphere, and that it is stimulated by sulfate amendment (Pester et al., 2012;
553 Wörner et al., 2016; Zecchin et al., 2018). In rice paddies, DSRB might have a crucial role in arsenic
554 thiolation by the production of sulfide, as previously observed for *Desulfovibrio desulfuricans* in the
555 human gut (DC. Rubin et al., 2014).
556 In the pore water of lower organic C Veronica soil, the obligate FeOB *Ferritrophicum*, responsible for
557 iron plaque formation in wetland plants (Weiss et al., 2007), positively responded to sulfate
558 fertilization. The significant increase of these iron-related microorganisms in sulfate amended rice
559 paddy mesocosms in the lower organic C soil contributes to explain the effect of sulfate amendment in
560 decreasing dissolved arsenic. Initially, this low organic C soil already had a significantly higher
561 abundance of DAsRB/DFeRB and DSRB in the rhizosphere soil and of FeOB in the pore water than
562 the high organic C soil. So, the more marked effect of sulfate amendment observed in Veronica pore
563 water in decreasing dissolved arsenic with respect to Fornazzo might be explained: 1) by a higher
564 immobilization of arsenic with enhanced iron plaque production by FeOB, similar to what was
565 previously described by Hu et al. (2007), and/or 2) by a higher co-precipitation or adsorption of
566 arsenite (produced by DAsRB), with secondary iron sulfide minerals (produced by DFeRB and DSRB)
567 or rather, as suggested before (Wang et al., 2020b), by mixed Fe(II)Fe(III) minerals (produced by
568 DFeRB coupled to re-oxidation of reduced sulfur). Moreover, the significant positive correlation
569 between SOB and total sulfur might contribute to explain the higher thiolation of arsenic in Veronica
570 pore water with respect to Fornazzo, providing locally higher concentrations of sulfide for arsenic
571 thiolation and supporting a more active sulfur cycling. It might be hypothesized that all the three
572 proposed microbial processes might have occurred in the mesocosms, resulting in the lower total

arsenic mobility and higher percentage contribution to total arsenic of thioarsenates in Veronica compared to Fornazzo. In the lower carbon soil, the presence of SOB and DSRB supports an active sulfur cycle fueled by available sulfur species. The positive correlation between SOB and thiolated arsenic species might be explained by the availability of S^0 and SO_4^{2-} to be used as electron acceptors by DSRB that produce sulfide and increment thiolation. On the other hand, in higher carbon soil the formation of FeS subtracts reduced sulfur from pore water thus establishing a less active microbially mediated sulfur cycle thus lowering thiolated arsenics.

In view of the upcoming water scarcity due to climate change, these outcomes suggest that a lower pore water content will likely have dramatic effects on element cycling mediated by microbial populations both present in the different soil/rhizosphere/water compartments and affected by the redox potential. It was previously shown (Zecchin et al., 2017a) that the microbial communities developed in the rhizosphere soil of rice cultivated under aerobic conditions were completely different if compared with the ones inhabiting the rhizosphere soil of continuously flooded rice plants. Hence, a lower water content in rice paddy soils is expected to progressively decrease the activity of pore water-specific microbial populations such as FeOB, to a level that will depend on the extent of water scarcity and/or to the type of water management adopted in the different agronomic schemes. Moreover, since rhizospheric microbial communities are significantly shaped by the pore water parameters, a decrease of soil water content might in general slow-down microbial arsenic, sulfur and iron cycling in rice paddies. This eventuality should be carefully considered by working on predictive models that include the outcomes of the present and all previous available data.

Overall, the data obtained in this study revealed that the compartment effect in rice paddy soil is the major driver of microbial diversity and functionality, ultimately affecting the complex interplay between microorganisms in rice paddy soil and arsenic, sulfur and iron mobility.

The microbial communities inhabiting the rhizosphere soil and the pore water developed over time,

were completely different and responded differently to sulfate amendment. In each compartment, soil type and C content significantly drove the development of the microbial communities, differentially sulfur- and iron-cycling microbial populations. The effect of sulfate amendment was also compartment-specific. It was linked to iron and sulfur concentrations and, in the low-C soil, promoted iron-oxidizing bacteria, dissimilatory arsenate-, iron- and sulfate-reducing bacteria, which were likely responsible for arsenic sequestration by secondary iron minerals and/or iron sulfides and its subsequent decrease in the pore water. The higher proportion of arsenic thiolation measured in sulfate-amended compared to unamended soil was found to be related to dissimilatory arsenate-, sulfate-reducing bacteria and sulfur-oxidizing bacteria.

These aspects should be considered carefully in the future, when the need to face a progressive drought due to climate change will necessarily lead to the adoption of more water-saving agronomic schemes in rice cultivation.

Funding

This study was supported by the University of Milano [PSR 2019 Linea 2 – Azione A, PSR 2021 Linea 6 1H_HUB project] and by Regione Lombardia [P-RICE project 23, d.d.s. 4403-28/03/2018].

Conflict of Interests

The authors declare no conflict of interests for this study.

References

Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol

2001;**26**(1):32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Arao T, Kawasaki A, Baba K et al. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environ Sci Technol 2009;**43**(24):9361-9367. https://doi.org/10.1021/es9022738

Anantharaman K, Brown CT, Hug LA et al. Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nat Commun 2016;**7**:13219. https://doi.org/10.1038/ncomms13219.

Anantharaman K, Hausmann B, Jungbluth SP et al. Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. ISME J 2018;**12**:1715–1728. https://doi.org/10.1038/s41396-018-0078-0

Arshad A, Dalcin Martins P, Frank J et al. Mimicking microbial interactions under nitrate-reducing conditions in an anoxic bioreactor: enrichment of novel Nitrospirae bacteria distantly related to *Thermodesulfovibrio*. Environ Microbiol 2017;**19**(12):4965-4977. https://doi.org/10.1111/1462-2920.13977

Bao Q, Huang Y, Wang F et al. Effect of nitrogen fertilizer and/or rice straw amendment on methanogenic archaeal communities and methane production from a rice paddy soil. Appl Microbiol Biotechnol 2016;**100**:5989–5998. https://doi.org/10.1007/s00253-016-7377-z

Bokulich NA, Kaehler BD, Rideout JR et al. Optimizing taxonomic classification of marker-gene amplicon sequences with qiime 2's q2-feature-classifier plugin. Microbiome 2018;**6**(1):90.

https://doi.org/10.1186/s40168-018-0470-z

Bolyen E, Rideout JR, Dillon MR et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;**37**(8):852-857. https://doi.org/10.1038/s41587-019-0209-9

Burdukiewicz M, Gagat P, Jabłoński, S et al. PhyMet2: a database and toolkit for phylogenetic and metabolic analyses of methanogens. Environ Microbiol Rep 2018;**10**(3):378-382. https://doi.org/10.1111/1758-2229.12648

Burton ED, Johnston SG, Kocar BD. Arsenic mobility during flooding of contaminated soil: the effect of microbial sulfate reduction. Environ Sci Technol 2014;**48**(23):13660-13667. https://doi.org/10.1021/es503963k

Callahan BJ, McMurdie PJ, Rosen MJ et al. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;**13**(7):581-583. https://doi.org/10.1038/nmeth.3869

Chao A. Non-parametric estimation of the number of classes in a population. Scand J Stat 1984;**11**:265–270.

Chen C, Li L, Huang K et al. Sulfate-reducing bacteria and methanogens are involved in arsenic methylation and demethylation in paddy soils. ISME J 2019;**13**(10):2523-2535. https://doi.org/10.1038/s41396-019-0451-7

668 Chen C, Yang B, Shen Y et al. Sulfate addition and rising temperature promote arsenic methylation and
669 the formation of methylated thioarsenates in paddy soils. Soil Biol Biochem
670 2021;**154**:108129. https://doi.org/10.1016/j.soilbio.2021.108129
671
672 Cho SR, Verma PP, Das S et al. A new approach to suppress methane emissions from rice cropping
673 systems using ethephon. Sci Tot Environ 2022;**804**:50159.
674 https://doi.org/10.1016/j.scitotenv.2021.150159
675
676 Colina Blanco AE, Kerl CF, Planer-Friedrich B. Detection of thioarsenates in rice grains and rice
677 products. J Agric Food Chem 2021;**69**(7):2287-2294. https://doi.org/10.1021/acs.jafc.0c06853
678
679 Crundwell FK. The dissolution and leaching of minerals: Mechanisms, myths and misunderstandings.
680 Hydrometallurgy 2013;**139**:132-148. https://doi.org/10.1016/j.hydromet.2013.08.003
681
682 Dahlawi S, Naeem A, Iqbal M et al. Opportunities and challenges in the use of mineral nutrition for
683 minimizing arsenic toxicity and accumulation in rice: a critical review. Chemosphere 2018;**194**:171-
684 188. https://doi.org/10.1016/j.chemosphere.2017.11.149
685
686 Dai J, Tang Z, Jiang N et al. Increased arsenic mobilization in the rice rhizosphere is mediated by iron-
687 reducing bacteria. Environ Pollut 2020;**263**:114561. https://doi.org/10.1016/j.envpol.2020.114561
688
689 Das S, Chou ML, Jean JS et al. Water management impacts on arsenic behavior and rhizosphere
690 bacterial communities and activities in a rice agro-ecosystem. Sci Tot Environ 2016;**542**:642– 652.
691 https://doi.org/10.1016/j.scitotenv.2015.10.122

692

693 Demoling F, Figueroa D, Bååth E. Comparison of factors limiting bacterial growth in different soils.
694 Soil Biol Biochem 2007;**39**:2485–2495. https://doi.org/10.1016/j.soilbio.2007.05.002

695

696 Ding LJ, Cui HL, Nie SA et al. Microbiomes inhabiting rice roots and rhizosphere. FEMS Microbiol
697 Ecol 2019;**95**(5):fiz040. https://doi.org/10.1093/femsec/fiz040

698

699 D. Lgs 152/2006. Norme in materia ambientale. Gazzetta Ufficiale 2006;88 (Supplemento Ordinario
700 96). https://www.gazzettaufficiale.it/dettaglio/codici/materiaAmbientale

701

702 Edwards JA, Santos-Medellín CM, Liechty ZS et al. Compositional shifts in root-associated bacterial
703 and archaeal microbiota track the plant life cycle in field-grown rice. PLoS biology
704 2018;**16**(2):e2003862. https://doi.org/10.1371/journal.pbio.2003862

705

706 Fan C, Liu G, Long Y et al. Thiolation in arsenic metabolism: a chemical perspective. Metallomics
707 2018;**10**(10):1368-1382. https://doi.org/10.1039/C8MT00231B

708

709 Fang X, Christl I, Blanco AEC et al. (2023). Decreasing arsenic in rice: Interactions of soil sulfate
710 amendment and water management. Environ Pollut 2023;**322**:121152.
711 https://doi.org/10.1016/j.envpol.2023.121152

712

713 Friedrich CG, Bardischewsky F, Rother D et al. Prokaryotic sulfur oxidation. Curr Opin Microbiol
714 2005;**8**:253–259. https://doi.org/10.1016/j.mib.2005.04.005

715

716 Griffith DM, Veech JA, Marsh CJ. Cooccur: probabilistic species co-occurrence analysis in R. J Stat
717 Softw 2016;**69**(2):1-17. https://doi.org/10.18637/jss.v069.c02
718
719 Halko N, Martinsson PG, Shkolnisky Y et al. An algorithm for the principal component analysis of
720 large data sets. arXiv e-prints. arXiv 2010;1007.5510
721
722 Hamady M, Knight R. Microbial community profiling for human microbiome projects: tools,
723 techniques and challenges. Genome Res 2009;**19**:1141–1152. https://doi.org/10.1101/gr.085464.108
724
725 Hamilton TL, Jones DS, Schaperdoth I et al. Metagenomic insights into S(0) precipitation in a
726 terrestrial subsurface lithoautotrophic ecosystem. Front Microbiol 2014;**5**:1–16.
727 https://doi.org/10.3389/fmicb.2014.00756
728
729 Herrmann M, Wegner CE, Taubert M et al. Predominance of *Cand*. Patescibacteria in groundwater is
730 caused by their preferential mobilization from soils and flourishing under oligotrophic conditions.
731 Front Microbiol 2019;**10**:1407. https://doi.org/10.3389/fmicb.2019.01407
732
733 Hossain M, Mestrot A, Norton GJ et al. Arsenic dynamics in paddy soil under traditional manuring
734 practices in Bangladesh. Environ Pollut
735 2021;**268**:115821. https://doi.org/10.1016/j.envpol.2020.115821
736
737 Hu ZY, Zhu YG, Li M et al. Sulfur (S)-induced enhancement of iron plaque formation in the
738 rhizosphere reduces arsenic accumulation in rice (*Oryza sativa* L.) seedlings. Environ Pollut
739 2007;147:387–393. https://doi.org/10.1016/j.envpol.2006.06.014

Huaidong HE, Waichin LI, Riqing YU et al. Illumina-based analysis of bulk and rhizosphere soil bacterial communities in paddy fields under mixed heavy metal contamination. Pedosphere 2017;**27**(3):569-578. https://doi.org/10.1016/S1002-0160(17)60352-7

Inagaki F, Takai K, Nealson KH et al. *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the ε-Proteobacteria isolated from Okinawa Trough hydrothermal sediments. Int J Syst Evol Microbiol 2004;**54**(5):1477-1482. https://doi.org/10.1099/ijs.0.03042-0

Inagaki F, Takai K, Kobayashi H et al. *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing ε-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. Int J Syst Evol Microbiol 2003;**53**(6):1801-1805. https://doi.org/10.1099/ijs.0.02682-0

Jia Y, Huang H, Chen Z et al. Arsenic uptake by rice is influenced by microbe-mediated arsenic redox changes in the rhizosphere. Environ Sci Technol 2014;**48**(2):1001-1007. https://doi.org/10.1021/es403877s

Jia Y, Bao P, Zhu YG. Arsenic bioavailability to rice plant in paddy soil: influence of microbial sulfate reduction. Journal Soils Sediments 2015;**15**:1960–1967. https://doi.org/10.1007/s11368-015-1133-3

Kato S, Shibuya T, Takaki Y et al. Genome-enabled metabolic reconstruction of dominant chemosynthetic colonizers in deep-sea massive sulfide deposits. Environ Microbiol 2018;**20**(2):862-877. https://doi.org/10.1111/1462-2920.14032

764

765 Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in
766 performance and usability. Mol Biol Evol 2013;**30**:772–780. https://doi.org/10.1093/molbev/mst010
767
768 Kodama Y, Watanabe K. *Sulfuricurvum kujiense* gen. nov., sp. nov., a facultatively anaerobic,
769 chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage
770 cavity. Int J Syst Evol Microbiol 2004;**54**(6):2297-2300. https://doi.org/10.1099/ijs.0.63243-0
771
772 Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species data.
773 *Oecologia* 2001;129(2):271-280. https://doi.org/10.1007/s004420100716
774
775 Legendre P, Legendre L. Numerical Ecology 2012. 3rd English Edition, Volume 24. Elsevier.
776
777 Li C, Carrijo DR, Nakayama Y et al. Impact of alternate wetting and drying irrigation on arsenic uptake
778 and speciation in flooded rice systems. Agric Ecosyst Environ 2019;**272**:188-198.
779 https://doi.org/10.1016/j.agee.2018.11.009
780
781 Liesack W, Schnell S, Revsbech NP. Microbiology of flooded rice paddies. FEMS Microbiol Rev
782 2000;**24**(5):625–645. https://doi.org/10.1111/j.1574-6976.2000.tb00563.x
783
784 Liu Q, Romani M, Wang J et al. Alternating wet–dry cycles rather than sulfate fertilization control
785 pathways of methanogenesis and methane turnover in rice straw-amended paddy soil. Environ Sci
786 Technol 2021;**55**(17):12075-12083. https://doi.org/10.1021/acs.est.1c03149
787

Lozupone CA, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005;**71**:8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Lynch JM, Whipps JM. Substrate flow in the rhizosphere. Plant Soil 1990;**129**:1–10. https://doi.org/10.1007/BF00011685

Ma R, Shen J, Wu J et al. Impact of agronomic practices on arsenic accumulation and speciation in rice grain. Environ Pollut 2014;**194**:217-223. http://dx.doi.org/10.1016/j.envpol.2014.08.004

Marschner P. Rhizosphere Biology 2011. Marschner's Mineral Nutrition of Higher Plants: Third Edition, 369–388. https://doi.org/10.1016/B978-0-12- 384905-2.00015-7

Meharg AA, Williams PN, Adomako E et al. Geographical variation in total and inorganic arsenic content of polished (white) rice. Environ Sci Technol 2009;**43**:1612–1617. https://doi.org/10.1021/es802612a

Meharg AA, Zhao FJ. Arsenic in rice grain. In Arsenic & Rice 2012, Springer, Dordrecht, pp. 11-30. https://doi.org/10.1007/978-94-007-2947-6

Moissl-Eichinger C, Pausan M, Taffner J et al. 2018. Archaea are interactive components of complex microbiomes. Trends Microbiol 2018;**26**(1):70-85. https://doi.org/10.1016/j.tim.2017.07.004

Oksanen J, Blanchet FG, Friendly M et al. vegan: Community Ecology Package. R package version 2.5-7 2020. https://CRAN.R-project.org/package=vegan

812

813 Parks DH, Chuvochina M, Waite DW et al. A standardized bacterial taxonomy based on genome
814 phylogeny substantially revises the tree of life. Nat Biotechnol 2018;36:996–1004.
815 https://doi.org/10.1038/nbt.4229

816

817 Pedregosa F, Varoquaux G, Gramfort A et al. Scikit-learn: machine learning in python. J Mach Learn
818 Res 2011;**12**:2825–2830.

819

820 Pester M, Knorr KH, Friedrich MW et al. Sulfate-reducing microorganisms in wetlands - fameless
821 actors in carbon cycling and climate change. Front Microbiol 2012;**3**:1–
822 19. https://doi.org/10.3389/fmicb.2012.00072

823

824 Pielou EC. The measurement of diversity in different types of biological collections. J Theoret Biol
825 1966;**13**:131–144.

826

827 Planer-Friedrich B, Hartig C, Lohmayer R et al. Anaerobic chemolithotrophic growth of the
828 haloalkaliphilic bacterium strain MLMS-1 by disproportionation of monothioarsenate. Environ Sci
829 Technol 2015;**49**(11):6554-6563. https://doi.org/10.1021/acs.est.5b01165

830

831 Price MN, Dehal PS, Arkin AP. FastTree 2 - Approximately maximum-likelihood trees for large
832 alignments. PLoS ONE 2010;**5**(3):e9490. https://doi.org/10.1371/journal.pone.0009490

833

834 R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical
835 Computing 2015, Vienna, Austria. http://www.R-project.org

836

837 Rago L, Cristiani P, Villa F et al. Influences of dissolved oxygen concentrationon biocathodic
838 microbial communities in microbial fuel cells. Bioelectrochemistry 2017;**116**:39–51.
839 https://doi.org/10.1016/j.bioelechem.2017.04.001

840

841 Revsbech NP, Pedersen O, Reichardt W et al. Microsensor analysis of oxygen and pH in the rice
842 rhizosphere under field and laboratory conditions. Biol Fertil Soils 1999;**29**:379-385.
843 https://doi.org/10.1007/s003740050568

844

845 Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression
846 analysis of digital gene expression data. Bioinformatics 2010;**26**:139–140.
847 https://doi.org/10.1093/bioinformatics/btp616

848

849 DC. Rubin SS, Alava P, Zekker I et al. Arsenic thiolation and the role of sulfate-reducing bacteria from
850 the human intestinal tract. Environ Health Perspect 2014;**122**(8);817-822.
851 http://dx.doi.org/10.1289/ehp.1307759

852

853 Said-Pullicino D, Miniotti EF, Sodano M et al. Linking dissolved organic carbon cycling to organic
854 carbon fluxes in rice paddies under different water management practices. Plant Soil 2016;**401**(1):273-
855 290. https://doi.org/10.1007/s11104-015-2751-7

856

857 Sekiguchi Y, Muramatsu M, Imachi H et al. *Thermodesulfovibrio aggregans* sp. nov. and
858 *Thermodesulfovibrio thiophilus* sp. nov., anaerobic, thermophilic, sulfate- reducing bacteria isolated
859 from thermophilic methanogenic sludge, and emended description of the genus *Thermodesulfovibrio*.

860 Int J Syst Evol Microbiol 2008;**58**:2541–2548. https://doi.org/10.1099/ijs.0.2008/000893-0

861

862 Sievert SM, Wieringa EB, Wirsen CO et al. Growth and mechanism of filamentous-sulfur formation by
863 *Candidatus* Arcobacter sulfidicus in opposing oxygen-sulfide gradients. Environ Microbiol
864 2007;**9**(1):271-276. https://doi.org/10.1111/j.1462-2920.2006.01156.x

865

866 Somenahally AC, Hollister EB, Loeppert RH et al. Microbial communities in rice rhizosphere altered
867 by intermittent and continuous flooding in fields with long-term arsenic application. Soil Biol Biochem
868 2011;**43**:1220-1228. https://doi.org/10.1016/j.soilbio.2011.02.011

869

870 St. John E, Flores GE, Meneghin J et al. Deep-sea hydrothermal vent metagenome-assembled genomes
871 provide insight into the phylum Nanoarchaeota. Environ Microbiol Rep 2019;**11**(2):262-270.
872 https://doi.org/10.1111/1758-2229.12740

873

874 Stolz JF, Oremland RS, Paster' BJ et al. *Sulfurospirillum*. Bergey's Manual of Systematics of Archaea
875 and Bacteria 2015;1-7. https://doi.org/10.1002/9781118960608.gbm01072

876

877 Stubner S, Wind T, Conrad R. Sulfur oxidation in rice field soil: activity, enumeration, isolation and
878 characterization of thiosulfate-oxidizing bacteria. Syst Appl Microbiol 1998;**21**(4):569-578.
879 https://doi.org/10.1016/S0723-2020(98)80069-6

880

881 Tan B, Foght J. Draft genome sequences of Campylobacterales (Epsilonproteobacteria) obtained from
882 methanogenic oil sands tailings pond metagenomes. Genome Announc 2014;2(5).
883 https://doi.org/10.1128/genomeA.01034-14

884

885 Tian W, Xiang X, Wang H. Differential impacts of water table and temperature on bacterial
886 communities in pore water from a subalpine peatland, central China. Front Microbiol 2021;**12**:649981.
887 https://doi.org/10.3389/fmicb.2021.649981
888
889 Umezawa K, Kojima H, Kato Y et al. *Dissulfurispira thermophila* gen. nov., sp. nov., a thermophilic
890 chemolithoautotroph growing by sulfur disproportionation, and proposal of novel taxa in the phylum
891 Nitrospirota to reclassify the genus *Thermodesulfovibrio*. Syst Appl Microbiol 2021;**44**(2):126184.
892 https://doi.org/10.1016/j.syapm.2021.126184
893
894 Umezawa K, Kojima H, Kato Y et al. Disproportionation of inorganic sulfur compounds by a novel
895 autotrophic bacterium belonging to Nitrospirota. Syst Appl Microbiol 2020;**43**(5):126110.
896 https://doi.org/10.1016/j.syapm.2020.126110
897
898 Veech JA. A probabilistic model for analysing species co-occurrence. Glob Ecol Biogeogr
899 2013;**22**(2):252-260. https://doi.org/10.1111/j.1466-8238.2012.00789.x
900
901 Wan S, Liao X, Zhou TT et al. Shift in archaeal community along a soil profile in coastal wheat-maize
902 rotation fields of different reclamation ages. Land Deg Dev 2021;**32**(15):4162-4173.
903 https://doi.org/10.1002/ldr.4022
904
905 Wang J, Halder D, Wegner L et al. Redox dependence of thioarsenate occurrence in paddy soils and the
906 rice rhizosphere. Environ Sci Technol 2020a;**54**(7):3940-3950. https://doi.org/10.1021/acs.est.9b05639
907

908 Wang J, Kerl CF, Hu P et al. Thiolated arsenic species observed in rice paddy pore waters. Nat Geosci
909 2020b;**13**(4):282-287. https://doi.org/10.1038/s41561-020-0533-1
910
911 Weiss JV, Rentz JA, Plaia T et al. Characterization of neutrophilic Fe (II)-oxidizing bacteria isolated
912 from the rhizosphere of wetland plants and description of *Ferritrophicum radicicola* gen. nov. sp. nov.,
913 and *Sideroxydans paludicola* sp. nov. Geomicrobiol J 2007;**24**(7-8):559-570.
914 https://doi.org/10.1080/01490450701670152
915
916 Wemheuer F, Taylor JA, Daniel R et al. Tax4Fun2: prediction of habitat-specific functional profiles
917 and functional redundancy based on 16S rRNA gene sequences. Environ Microbiome 2020;**15**:1-12.
918 https://doi.org/10.1186/s40793-020-00358-7
919
920 Wörner S, Zecchin S, Dan J et al. Gypsum amendment to rice paddy soil stimulated bacteria involved
921 in sulfur cycling but largely preserved the phylogenetic composition of the total bacterial community.
922 Environ Microbiol Rep 2016;**8**(3):413-423. https://doi.org/10.1111/1758-2229.12413
923
924 Xu XY, McGrath SP, Meharg AA et al. Growing rice aerobically markedly decreases arsenic
925 accumulation. Environ Sci Technol 2008;**42**(15):5574-5579. https://doi.org/10.1021/es800324u
926
927 Xu X, Wang P, Zhang J et al. Microbial sulfate reduction decreases arsenic mobilization in flooded
928 paddy soils with high potential for microbial Fe reduction. Environ Pollut 2019;**251**:952-960.
929 https://doi.org/10.1016/j.envpol.2019.05.086
930
931 Yang YP, Tang XJ, Zhang HM et al. The characterization of arsenic biotransformation microbes in

paddy soil after straw biochar and straw amendments. J Hazard Mater 2020;**391**:122200. https://doi.org/10.1016/j.jhazmat.2020.122200

Yang YP, Zhang HM, Yuan HY et al. Microbe mediated arsenic release from iron minerals and arsenic methylation in rhizosphere controls arsenic fate in soil-rice system after straw incorporation. Environ Pollut 2018;**236**:598-608. https://doi.org/10.1016/j.envpol.2018.01.099

Ye R, Horwath WR. Influence of rice straw on priming of soil C for dissolved organic C and CH_4 production. Plant Soil 2017;**417**(1):231-241. https://doi.org/10.1007/s11104-017-3254-5

Zecchin S, Colombo M, Cavalca L. Exposure to different arsenic species drives the establishment of iron-and sulfur-oxidizing bacteria on rice root iron plaques. World J Microbiol Biotechnol 2019;**35**(8):1-12. https://doi.org/10.1007/s11274-019-2690-1

Zecchin S, Corsini A, Martin M et al. Influence of water management on the active root-associated microbiota involved in arsenic, iron, and sulfur cycles in rice paddies. Appl Microbiol Biotechnol 2017a;**101**:6725–6738. https://doi.org/10.1007/s00253-017-8382-6

Zecchin S, Corsini A, Martin M et al. Rhizospheric iron and arsenic bacteria affected by water regime: Implications for metalloid uptake by rice. Soil Biol Biochem 2017b;**106**:129-137. http://dx.doi.org/10.1016/j.soilbio.2016.12.021

Zecchin S, Mueller RC, Seifert J et al. Rice Paddy *Nitrospirae* carry and express genes related to sulfate respiration: proposal of the new genus "*Candidatus* Sulfobium". Appl Environ Microbiol

2018;**84**(5):e02224-17. https://doi.org/10.1128/AEM .02224-17

Zheng MZ, Cai C, Hu Y et al. Spatial distribution of arsenic and temporal variation of its concentration in rice. New Phytol 2011;**189**:200–209. https://doi.org/10.1111/j.1469-8137.2010.03456.x

Zhou W, Wan M, He P et al. Oxidation of elemental sulfur in paddy soils as influenced by flooded condition and plant growth in pot experiment. Biol Fertil Soil 2002;**36**(5):384-389. https://doi.org/10.1007/s00374-002-0547-4

Zou L, Zhang S Duan D et al. Effects of ferrous sulfate amendment and water management on rice growth and metal(loid) accumulation in arsenic and lead co-contaminated soil. Environ Sci Pollut Res 2018;**25**(9):8888-8902. https://doi.org/10.1007/s11356-017-1175-8