

# Subaqueous soils and preliminary considerations on the occasional formation of "fairy circles" in the Comacchio saline (Province of Ferrara, Italy)

Stefano Cremonini<sup>1</sup>, William Trenti<sup>2\*</sup>, Roberta Pastorelli<sup>3</sup>, Arturo Fabiani<sup>3</sup>, Gilmo Vianello<sup>4</sup>, Livia Vittori Antisari<sup>2</sup>

<sup>1</sup> PAM, University of Bologna, Italy

<sup>2</sup> Department of Agricultural and Food Sciences, University of Bologna, Italy

<sup>3</sup> Research Centers Agriculture and Environment, CREA, Firenze, Italy

<sup>4</sup> National Academy of Agriculture, Italy

\* Corresponding author E-mail: [william.trenti@unibo.it](mailto:william.trenti@unibo.it)

## Article info

Received 22/6/2024; received in revised form 28/6/2024; accepted 1/7/2024

DOI: 10.6092/issn.2281-4485/19883

© 2024 The Authors.

## Abstract

The results of a survey carried out inside the salt basins of the Comacchio Saline (Province of Ferrara, Italy) are reported, occasionally affected by the formation of numerous submerged circular shapes of approximately two meters in diameter and whose crowns quickly tend to darken. A rare phenomenon that has so far only been reported in China in the Shanghai swamps, which have been called "fairy circles". The coordination of pedological and microbiological investigations on soils that are permanently submerged (Typic Sulfiwassents) or occasionally emerged (Aeric Sulfiwassents) has highlighted how the formation of the "fairy circles" of the Comacchio salt pans can be attributed to concomitant exogenous conditions (increase in temperatures and salinity of water) and endogenous (gas emissions of methane and sulfur dioxide), which is followed by intense bacterial activity and proliferation of microalgae which explain the darkening of the crowns. It was also possible to highlight a different distribution of bacteria along the profiles with dominance of methanogens in the epipedon and of sulfur reducers below it.

## Keywords

*fairy circles, hydromorphic and subaqueous soils, methanogenic bacteria, sulphite and sulphate reducing bacteria.*

## Location of the area, saline structure and representation of the "fairy circles" phenomenon

The Saline di Comacchio (Province of Ferrara), now a protected area of over 600 hectares in the Po Delta Park, preserve the structures of the last plant built in the Napoleonic era for the production of salt and kept in operation until 1984 (Fig. 1). Unlike the neighboring areas which are mostly in contact with the marine waters of the Adriatic, the underwater soils of the salt pan are characterized by conductivity values frequently  $>15 \text{ mS cm}^{-1}$ , and by a significant presence of sulphides, conditions which limit the proliferation of species halophilous plants and, conse-

quently, the deposition of organic material on the bottom of the historic tanks. Spring-summer satellite images (GOOGLE-Earth) of 2014 and 2020 highlighted the formation of numerous circular shapes on the bottom of the tanks, especially in the north-eastern part of the salt pans (Fig. 2). The circles are characterized in the form of a circular crown with a diameter of approximately two meters slightly raised both at the center and at the edges, thus forming a slight internal depression. The crown of each circle tends to darken rapidly after a few hours after its formation, highlighting the development of an intense biological activity.

**Figure 1**

*In the center of the image, the hatched perimeter of the Comacchio Saline. The star symbol locates the area shown in figure 2.*



**Figure 2.** *Satellite image from April 2020 of an area located within the Comacchio Saline affected by the formation of submerged circles «Fairy circles»*

The trigger of the phenomenon was hypothesized to be concomitant with specific climatic conditions: absence of precipitation for periods exceeding 60 days, intense evaporation and high salt concentration of the water in the tanks, high air temperatures even at night. Similar phenomena are found in the coastal saline marshes near Shanghai in eastern China, where

the circles called fairy circles also reflect a transitory phenomenon (Cantwell, 2021; Zhao et al., 2021). The recurrence of the phenomenon in the spring of 2020 allowed for careful observation with the localization and delimitation of the affected areas and in 2021, after having obtained the necessary authorizations from the Delta Regional Park, the precise observation and sampling activity began through the opening of profiles and surveys in the submerged areas and also in some emerged ones.

## **Materials and Methods**

### **Pedological survey and sampling**

In submerged conditions, surveys were carried out at variable depths by sampling both the submerged soil and the overlying water column using a Beeker sampler (Eijkelkamp, NL) equipped with a polyethylene tube (6 cm Ø diameter) (Ferronato et al., 2016). Then taking advantage of the temporary emptying of some tanks for maintenance, it was possible to open some profiles and at the same time carry out drillings up to a depth of 3.5 m from the ground level in correspondence with some circles. The reading of the soil characteristics was carried out on the extruded columns in the case of submerged soils and on profiles or on their reconstruction from drilling in the case of emerged soils. The sampling was carried out on each of the horizons of the various types of profiles.



**Figura 3.** Localization of sampling sites

### Analytical methodologies on soil samples

Soil samples were air dried and sieved to 2 mm. Texture was determined by the pipette method after dispersing the soil samples with sodium hexameta-phosphate solution. Soil pH and electrical conductivity were measured on a 1:2.5 (w:v) soil:deionized water suspension, while total carbonates ( $\text{CaCO}_3$ ) were quantified by volumetric method. For the detection of total organic carbon (TOC), the soil samples were finely ground into powder, treated with 2M HCl to eliminate carbonates and finally processed with an elemental analyzer. For sulfides, the semi-quantitative SPS (Sulfide Paper Sensor) method was used (Pellegrini et al., 2018). For other characterizations, please refer to the guidelines of the National Soil Survey Center USDA (Schoeneberger et al., 2012).

### Sampling and microbiological analytical methodologies

Using plexiglass liners, two columns of underwater soil were collected respectively in the central and peripheral parts of a submerged circle (Fig. 4). The analysis of the microbial communities was conducted on samples collected at increasing depth in each of the two columns.

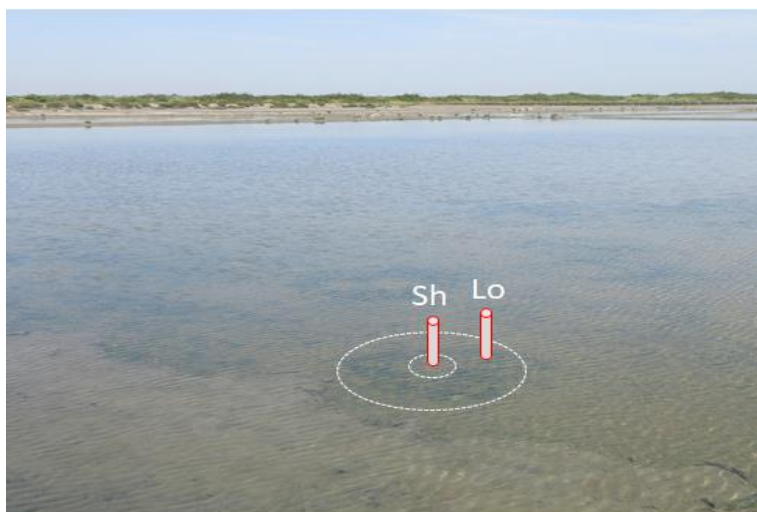
In the central column (short core, Sh) three different horizons were identified at 0-0.5 (Sh1), 0.5-4.0 (Sh2) and 4.0-16.5 (Sh3) cm depth while in the peripheral one (long core, Lo) a white superficial layer (Lo0) and three different horizons were identified at 0-1.5 (Lo1), 1.5-4.0 (Lo2) and 4-28

(Lo3) cm deep. Double strand DNA (dsDNA) was extracted from 0.5 g of soil using the Fast DNA Spin Kit for soil (3 replicates per horizon). The concentration and purity of DNA extracts were determined using a NanoDrop Lite Plus spectro-photometer (Thermo Fisher Scientific, Wilmington, NC, USA). Total DNA concentration was used as proxy of soil microbial biomass.

The composition and structure of the bacterial and archaeal communities were determined by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) using 16S rRNA gene-specific primer pairs (GC986F/Uni1401R and 357F/691R; Nübel et al., 1996, Watanabe et al., 2004) as previously described (Pastorelli et al., 2020). The quantification of bacteria, archaea, anaerobi fungi, and some microbial groups involved in methane ( $\text{CH}_4$ ) production and sulfur (S) metabolism was conducted by real-time PCR (Pastorelli et al., 2021) using the specific primer reported in Table 1.

**Table 1.** Primer pairs and annealing temperature used for real time PCR absolute quantification of the different microbial

Microbial group	Target gene	Primer	Sequence 5'-3'	Reference
Bacteria	16S rRNA	Bac341F	CCTACGGGAGGCAGCAG	Muyzer et al., 2009
		Bac805R	GGACTACHVGGGTWTCTAA	Caporaso et al., 2011
Archaea	16S rRNA	Arc344F	ACGGGGYGCAGCAGGCGCGA	Raskin et al., 1994
		Arc806R	GGACTACVSGGGTATCTAAT	Takai and Horikoshi, 2000
Anaerobic fungi	28S rRNA	AF-LSU-F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	Dollhofer et al., 2016
		AF-LSU-R	CTTGTTAAMYRAAAAAGTGCATT	
Methanogenic archaea	mcrA	qmcrAF	TTCGGTGGATCDCARAGRGC	Denman et al., 2007
		qmcrAR	GBARRTCGWAWCCGTAGAAWCC	
Sulfite-reducing bacteria	dsrA	DSR1F	ACSCACTGGAAGCACG	Foti et al., 2007
		DSRR	GTGGMRCCGTGCAKRTTG G	
Sulfate-reducing bacteria	aprA	AprA-1-FW	TGGCAGATCATGATYMAYGG	Deplancke et al., 2000
		AprA-5-RV	GCGCCAACYGGRCRTA	



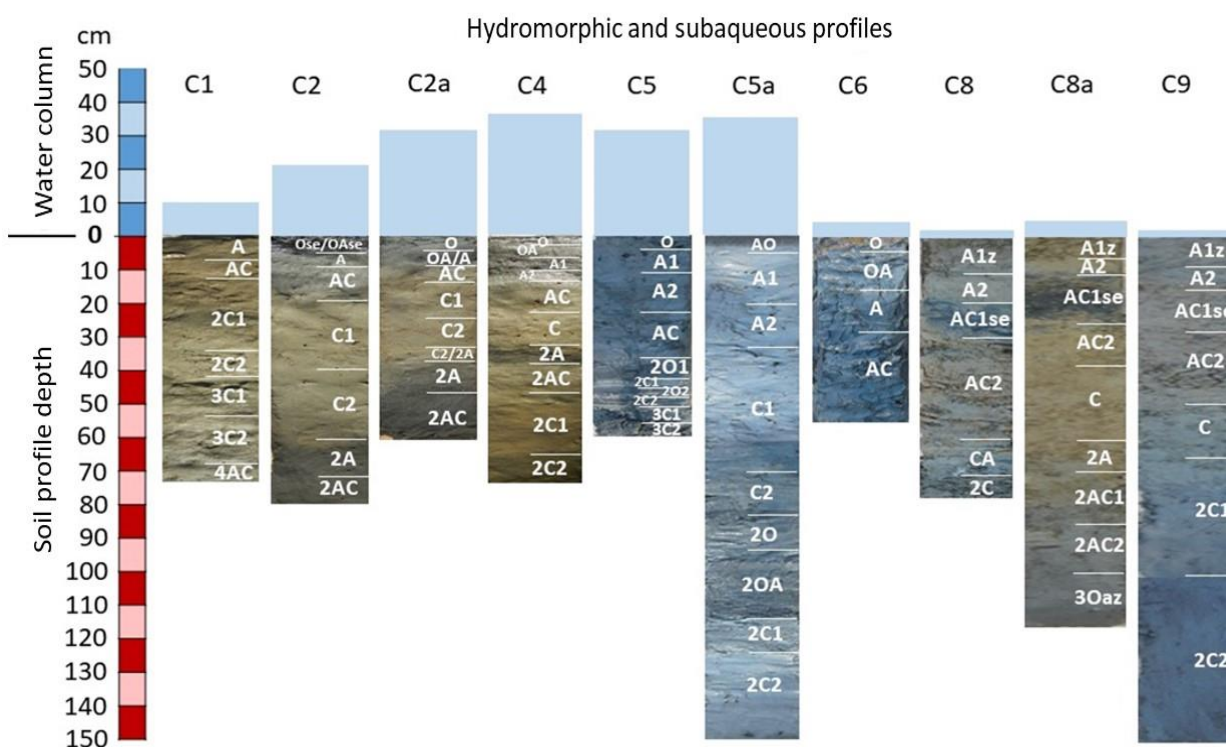
**Figure 4**  
Submerged circles with localization of samples using a column sampler for microbiological analyses

**Results and Discussion**

**Chemical-physical characteristics and classification of soils**

The subaqueous soils present inside the tanks are characterized by conductivity values mostly >15 mS cm<sup>-1</sup> and by significant concentrations of sulphides (Ase and Acse horizons), a situation which is accentuated in the site further south-east where in hot periods the low level of the water column favors evaporation with consequent saline saturation in the first levels of the underwater soils (Az Horizons) (Ta-

ble 2). These conditions limit the proliferation of plant species, including halophilic ones, and, consequently, the deposition of organic material on the bottom of the tanks. In accordance with the Soil Taxonomy (SSS, 2022) the soils of profiles C8 and C8a fall into the subgroup of Typic Sulfiwassents, C6 and C9 in that of Aeric Sulfiwassents and C2, C2a, C3 in that of Sulfic Haplowassents. In the other cases (profiles C4, C5 and C5a) the greater exchange with marine waters decreases the salt concentration (Typic Haplowassents) and there is no formation of submerged circles (Fig. 5).



**Figure 5.** Representation of the hydromorphic and subaqueous profiles in the investigation area

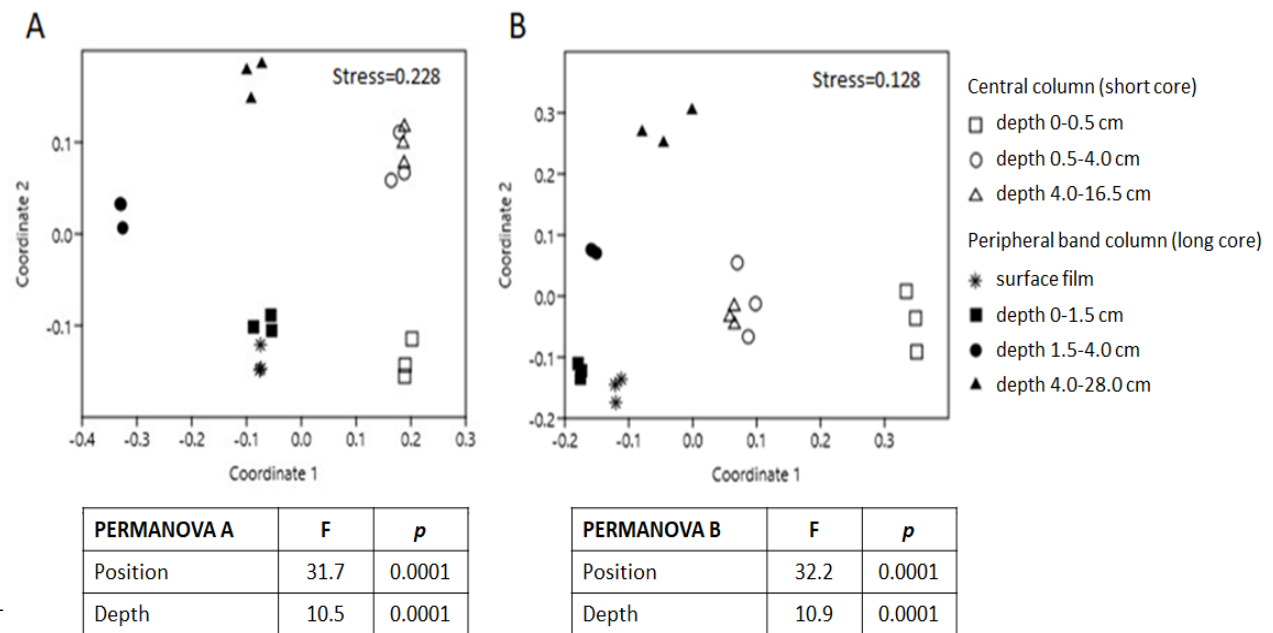
**Table 2.** *Soil chemical-physical characteristics*

Soil profile	Horizon	Depth cm	pH (H <sub>2</sub> O)	C.E. mS cm <sup>-1</sup>	CaCO <sub>3</sub> tot g kg <sup>-1</sup>	Texture			TOM %	TOC g kg <sup>-1</sup>
						Sand Ø 2000-50 µ g kg <sup>-1</sup>	Silt Ø 50-2 µ g kg <sup>-1</sup>	Clay Ø <2 µ g kg <sup>-1</sup>		
C1	A	0_5	8,66	12,4	170,9	297	492	211	2,86	15,9
	AC	5_12	8,58	13,9	146,5	282	452	266	2,27	12,6
	2C1	12_34	8,49	12,7	155,4	55	560	385	3,03	16,8
	2C2	34_42	8,47	13,3	182,0	61	629	310	2,16	12,0
	3C1	42_55	8,53	11,9	159,8	99	566	335	2,26	12,6
	3C2	55_70	8,44	13,7	162,1	84	527	389	2,70	15,0
	4AC?	70_73	8,26	18,5	146,5	53	369	578	1,62	9,00
C2	Ose	0_3	8,43	7,76	182,0	218	282	500	8,88	49,3
	OAse	3_5	8,48	9,04	173,2	149	523	328	3,06	17,0
	A	5_9	8,53	9,66	164,3	201	507	292	2,35	13,1
	AC	9_19	8,79	8,56	188,7	48	657	295	1,72	9,06
	C1	19_40	8,60	12,5	182,0	65	414	521	2,72	15,1
	C2	40_62	8,43	16,2	157,6	65	439	496	3,35	18,6
	2A	62_71	8,37	16,6	159,8	51	410	539	3,39	18,8
2AC	71_80	8,41	17,7	173,2	69	628	303	2,71	15,1	
C2a	O	0_2	8,48	9,80	186,5	200	384	416	5,15	28,6
	OA>A	2_8	8,61	8,07	173,2	190	433	377	2,33	12,9
	AC	8_12	8,66	9,09	159,8	168	339	493	2,35	13,1
	C1	12_24	8,64	9,88	153,2	267	567	166	2,45	13,6
	C2	24_33	8,66	11,7	151,0	214	212	574	1,35	7,50
	C2>2A	33_38	8,49	12,7	111,0	346	342	312	3,08	17,1
	2A	38_48	8,53	14,0	166,5	275	237	488	2,83	15,7
2AC	48_53	8,17	16,6	153,2	225	235	540	3,27	18,2	
C4	O	0_1	8,31	6,56	151,0	343	412	245	2,38	13,2
	OA	1_5	8,31	9,27	142,1	398	280	322	2,71	15,1
	A1	5_10	8,37	8,46	124,3	397	169	434	2,24	12,4
	A2	10_13	8,39	8,03	111,0	462	379	159	1,86	10,3
	AC	13_22	8,55	7,36	115,4	358	415	227	1,66	9,02
	C	22_33	8,52	8,02	111,0	195	553	252	1,73	9,06
	2A	33_38	8,52	7,95	117,7	322	217	461	2,33	12,9
	2AC	38_47	8,49	7,34	111,0	263	269	468	2,01	11,2
	2C1	47_66	8,51	7,65	122,1	318	393	289	1,70	9,41
	2C2	66_73	8,52	9,03	117,7	288	428	284	1,59	8,80
C8	Az	0-3	8,43	20,39	223,3	90	702	208	1,60	9,21
	A	3-15/17	8,55	8,23	168,7	91	644	265	4,87	27,1
	AC1se	15/17-24	8,44	9,24	175,4	109	761	130	3,12	17,3
	AC2	24-48	8,44	10,66	166,5	98	732	170	4,73	26,3
	CA	48-55	8,27	15,45	151,0	159	619	222	4,40	24,4
	2C	55-60	8,38	14,19	158,5	254	587	159	2,80	15,6
C8bis	A1z	0-3	8,43	20,28	218,8	109	733	158	1,60	9,21
	A2	3-8	8,52	7,69	165,6	85	763	152	8,03	44,6
	AC1se	8-17	8,38	10,66	186,5	104	732	164	4,55	20,2
	AC2	17-25	8,49	10,06	179,8	105	763	132	4,01	25,3
	C	25-47,5	8,42	11,06	155,4	101	703	196	3,00	16,6
	2A1	47,5-63,5	8,32	13,85	149,6	261	465	274	6,56	36,5
	2A2	63,5-86	7,91	14,77	133,6	188	589	223	7,14	39,7
	2AC1	86-114	7,97	15,72	157,6	226	545	229	6,19	34,4
	3Oaz	114-172	8,15	20,86	135,4	149	559	292	4,38	24,3
	3Oa	172-209	7,97	18,71	122,1	155	599	246	8,85	49,2
	3A	209-252	8,00	17,12	122,5	209	541	250	6,54	36,3
	4AC1	252-288	8,08	13,27	124,3	474	339	187	4,34	24,1
4AC2	288-325	8,04	13,72	131,0	551	271	178	5,61	31,2	
C9	Az	0-6	8,50	20,85	165,2	71	758	171	1,68	9,40
	A	6-15	8,15	8,41	172,8	102	691	207	8,36	46,5
	AC1se	15-30	8,50	10,62	161,2	107	679	214	3,91	21,7
	AC2	30-42	8,39	11,01	144,3	82	702	216	7,56	42,0
	C	42-59	8,43	14,63	155,8	99	699	202	2,57	14,3
	2C1	59-86	8,13	17,84	134,5	255	529	216	3,85	21,4
	2C2	86-115	8,04	17,96	131,0	249	583	168	3,13	17,4

### Soil microbial communities

Both the position in the submerged circle (central or peripheral band) and the depth have a significant influence on the composition and structure of the

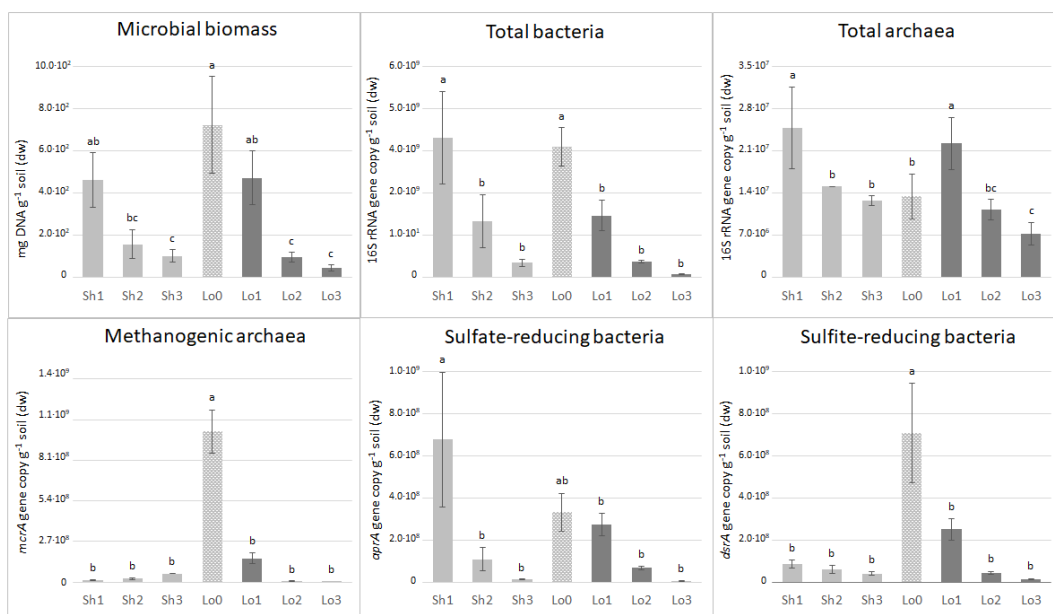
bacterial and archaeal communities in the soil (Fig. 6). In general, the total biomass (calculated in terms of µg DNA g<sup>-1</sup> dry weight (dw) soil) and the abundance of bacteria and archaea (calculated in terms of



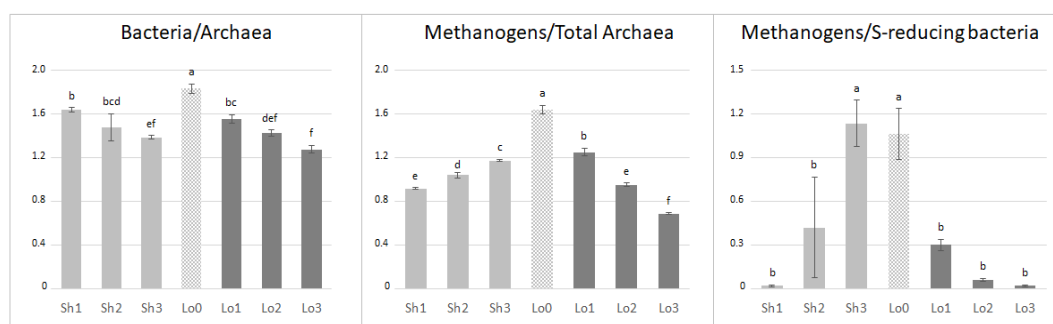
**Figure 6.** nMDS and PERMANOVA analyses performed on PCR-DGGE profiles obtained by amplification of the bacterial (A) and archaeal (B) 16S rRNA gene.

16S rRNA gene copy number  $g^{-1}$  dry weight (dw) soil) decrease significantly with depth (Fig. 7). The real time PCR conducted with primer pairs for 28S rRNA of anaerobic fungi showed values below the detection threshold. The *mcrA* gene, used as a marker gene to quantify the group of methanogenic archaea, shows a peak in the whitish film present on the surface of the peripheral profile, while based on the depth it shows an opposite trend in the two different soil profiles. Similarly to total bacteria, S reducers

also decrease with depth (Fig. 7). Bacteria resulted more abundant than archaea in the surface layers of the soils. In contrast, archaeal communities dominate the deeper layers (Fig. 8). Furthermore, the results obtained displayed distinct trends of methanogens in the central and two peripheral columns (Fig. 8). Compared to total archaea and S-reducing bacteria, methanogenic archaea dominate the deepest layer of the central zone of the "fairy circles" and the surface film of the peripheral zone.



**Figure 7**  
Total biomass (calculated in terms of mg of dsDNA extracted per g of soil) and abundance of microbial communities (calculated in terms of marker gene copy number per g of soil). Sh, short core (central column); Lo, long core (peripheral column)



**Figure 8**  
Relationships between groups of microorganisms versus depth diversity in the submerged soil sample. Sh, short core (central column); Lo, long core (peripheral column)

Oxygen levels typically decrease with soil depth due to microbial respiration and limited diffusion. Such variations create distinct niches, leading to the distribution of different microbial communities across soil profiles according to their ecological requirements (Naylor et al., 2022). Sulfur bacteria and methanogens have similar nutritional requirements and compete with each other for the use of substrates such as  $H_2$ ,  $CO_2$  and  $CH_3COO^-$  (Stams et al., 2005). In the central zone of the submerged circle, deeper layers may provide favorable conditions for methanogen abundance and activity, possibly due to higher organic matter content and anaerobic conditions (Liu and Whitman, 2008). Conversely, the peripheral zone may support methanogen activity in surface layers due to different hydrological dynamics and nutrient availability while, in deeper layers, S-reducing bacteria may outcompete methanogens for substrates, thereby limiting  $CH_4$  production and release (Whitman et al., 2006). Based on these indications, it would seem conceivable that the genesis of the "circles" is linked to a progressive concentration over time of bacterial methane developed in the first "organic" horizon, buried 10-15 cm deep beneath the bottom of the saline: upon reaching the threshold of pressure necessary for the vertical exfiltration of the gas, the small local reservoir decompresses, creating the annular depression peripheral to the central bulb constituting the scar of the conduit for the escape of the gas and associated fluids.

## Conclusions

The pedological, chemical-physical and microbiological investigations conducted on the submerged soils of some salt pans lead to support the hypothesis that the origin of the submerged circles ("fair circles") can be attributed to particular

and concomitant endogenous conditions (methane gaseous emissions and sulphides) and exogenous (climatic, in particular). The initial circular morphology assumed due to the emergence of gaseous bubbles is immediately followed by a rapid proliferation of microalgae, methanobacteria and other colonies of bacteria, including sulphurous ones which give the crown its typical dark colour. The PCR-DGGE analysis revealed that both the position in the "fair circle" and the depth of the sample significantly influence the structure and composition of the soil bacterial and archaeal communities. Total biomass and abundance of bacteria and archaea decrease significantly with depth. The *mcrA* gene, used as a marker gene to quantify the group of methanogenic archaea, shows a peak of the latter in the whitish film present on the external surface of the crown and an increase with depth at the central umbo. Sulfur bacteria and methanogens have similar nutritional needs and compete with each other for the use of substrates such as  $H_2$ ,  $CO_2$  and  $CH_3COO^-$ , with the result that methanogens dominate in the most superficial layer of submerged soil and sulfur reducers in the deeper layers. As confirmed by the presence of ACse horizons of significant thickness in the Sulfiwassents. The hypothesis has been put forward that the progressive concentration of bacterial methane accumulated over time in the organic and organo-mineral epipedons buried in the first meters of depth of the salt pans, once the necessary pressure threshold is reached, would produce the vertical exfiltration of the gas with consequent formation of the peripheral annular depression.

## Acknowledgments

The authors thank the Direction of Comacchio Saline for having granted the monitoring and sampling of subaqueous soils

## References

- CANTWELL M. (2021) How fairy circles form in Shanghai's salt marshes. *Plants & Animals*. <https://doi.org/10.1126/science.abh2247>
- CAPORASO J.G., LAUBER C.L., WALTERS W.A., BERG-LYONS D., LOZUPONE C.A., TURNBAUGH P.J., FIERER N., KNIGHT R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108:4516-4522. <https://doi.org/10.1073/pnas.1000080107>
- DENMAN S.E., TOMKINS N.W., MCSWEENEY C.S. (2007) Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiology Ecology* 62:313-322. <https://doi.org/10.1111/j.1574-6941.2007.00394.x>
- DEPLANCKE B., HRISTOVA K.R., OAKLEY H.A., MCCRACKEN V.J., AMINOV R., MACKIE R.I., GASKINS H.R. (2000) Molecular ecological analysis of the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. *Applied and Environmental Microbiology*, 66(5):2166-2174. <https://doi.org/10.1128/AEM.66.5.2166-2174.2000>
- DOLLHOFFER V., CALLAGHAN T.M., DORN-IN S., BAUER J., LEBUHN M. (2016) Development of three specific PCR-based tools to determine quantity, cellulolytic transcriptional activity and phylogeny of anaerobic fungi. *Journal of Microbiological Methods* 127:28-40. <https://doi.org/10.1016/j.mimet.2016.05.017>
- FERRONATO C., FALSONE G., NATALE M., ZANNONI D., BUSCAROLI A., VIANELLO G., VITTORI ANTISARI L. (2016) Chemical and pedological features of subaqueous and hydromorphic soils along a hydrosequence within a coastal system (San Vitale Park Northern Italy). *Geoderma*, 265:141-151. <https://doi.org/10.1016/j.geoderma.2015.11.018>
- FOTI M., SOROKIN D.Y., LOMANS B., MUSSMAN M., ZACHAROVA E.E., PIMENOV N.V., KUENEN J.G., MUYZER G. (2007) Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. *Applied and Environmental Microbiology* 73(7):2093-2100. <https://doi.org/10.1128/AEM.02622-06>
- LIU Y., WHITMAN W.B. (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, 1125(1):171-189. <https://doi.org/10.1196/annals.1419.019>
- MUYZER G., DE WAAL E.C., UITTERLINDEN A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59:695-700. <https://doi.org/10.1128/aem.59.3.695-700.1993>
- NAYLOR D., MCCLURE R., JANSSON J. (2022) Trends in microbial community composition and function by soil depth. *Microorganisms*, 10(3):540. <https://doi.org/0.3390/microorganisms10030540>
- NÜBEL U., ENGELEN B., FELSKA A., SNAIDR J., WIESHUBER A., AMANN R.I., LUDWIG W., BACKHAUS H. (1996) Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology*, 178(19):5636-5643. <https://doi.org/10.1128/jb.178.19.5636-5643.1996>
- PASTORELLI R., PALETTO A., AGNELLI A.E., LAGOMARSINO A., DE MEO I. (2020) Microbial communities associated with decomposing deadwood of downy birch in a natural forest in Khibiny Mountains (Kola Peninsula, Russian Federation). *Forest Ecology and Management*, 455:117643. <https://doi.org/10.1016/j.foreco.2019.117643>
- PASTORELLI R., PALETTO A., AGNELLI A.E., LAGOMARSINO A., DE MEO I. (2021) Microbial diversity and ecosystem functioning in deadwood of black pine of a temperate forest. *Forests*, 12(10):1418. <https://doi.org/10.3390/f12101418>
- PELLEGRINI E., CONTIN M., VITTORI ANTISARI L., VIANELLO G., FERRONATO C., DE NOBILI M. (2018) A new paper sensor method for field analysis of acid volatile sulfides in soils. *Environmental Toxicology and Chemistry*, 37:3025-3031. <https://doi.org/10.1002/etc.4279>
- RASKIN L., STROMLEY J.M., RITTMANN B.E., STAHL D.A. (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Applied and Environmental Microbiology*, 60:1232-1240. <https://doi.org/10.1128/aem.60.4.1232-1240.1994>
- ROGAN B., LEMKE M., LEVANDOWSKY M., GORREL T. (2005) Exploring the sulfur nutrient cycle using the Winogradsky column. *The American Biology Teacher*, 67(6):348-356. [https://doi.org/10.1662/0002-7685\(2005\)067](https://doi.org/10.1662/0002-7685(2005)067)
- SCHOENEBERGER P.J., WYSOCKI D.A., BENHAM E.C., and SOIL SURVEY STAFF (2012) Field book for describing and sampling soils. Version 3.0. National Resources Conservation Service, National Soil Survey Center, Lincoln, NE. ISBN 978-0-16-091542-0



SSS - SOIL SURVEY STAFF (2022) Keys to Soil Taxonomy, 13th edition. USDA Natural Resources Conservation Service.

STAMS A.J.M., PLUGGE C.M., DE BOK F.A., VAN HOUTEN B.H.G.W., LENS P., DIJKMAN H., WEIJMA J. (2005) Metabolic interactions in methanogenic and sulfate-reducing bioreactors. *Water Science and Technology*, 52(1-2):13-20. <https://doi.org/10.2166/wst.2005.0493>

TAKAI K.E.N., HORIKOSHI K. (2000) Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Applied and Environmental Microbiology*, 66:5066-5072. <https://doi.org/10.1128/AEM.66.11.5066-5072.2000>

TAO P., TAN K., KE T., LIU S., ZHANG W., YANG J., ZHU X. (2022) Recognition of ecological vegetation

fairy circles in intertidal salt marshes from UAV LiDAR point clouds. *International Journal of Applied Earth Observation and Geoinformation*, 114:103029. <https://doi.org/10.1016/j.jag.2022.103029>

WATANABE T., ASAKAWA S., NAKAMURA A., NAGAOKA K., KIMURA M. (2004) DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. *FEMS Microbiology Letters*, 232(2):153-163. [https://doi.org/10.1016/S0378-1097\(04\)00045-X](https://doi.org/10.1016/S0378-1097(04)00045-X)

ZHAO L.X., ZHANG K., SITEUR K., LI X.-S., LIU Q.-X., VAN DE KOPPEL J.(2021) Fairy circles reveal the resilience of self-organized salt marshes. *Science Advances*, 7(6):1-12. <https://doi.org/10.1126/sciadv.abe1100>