Feasibility of a *soft* biological improvement of natural soils used in compacted linear earth construction

Laura Morales · Enrique Romero · Cristina Jommi ·
Eduardo Garzón · Antonio Giménez

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L. Morales · E. Garzón · A. Giménez
Departamento de Ingenieria, Universidad de Almería, carretera
Sacramento s/n, Cañada de San Urbano, 04120 Almería, Spain
e-mail: mhl274@ual.es; egarzon@ual.es; agimfer@ual.es

E. Romero (✉)
Department of Geotechnical Engineering and Geosciences,
Universitat Politècnica de Catalunya, c/Jordi Girona 1-3,
Campus Nord UPC, Edificio D-2, 08034 Barcelona, Spain
e-mail: enrique.romero-morales@upc.edu

C. Jommi
Department of Geoscience and Engineering, Faculty of Civil
Engineering and Geoscience, Delft University of Technology,
Stevinweg 1, 2628 CN Delft, The Netherlands
e-mail: c.jommi@tudelft.nl

1 Introduction

Linear earthworks, such as levees, railway and road embankments, are nowadays a political priority due to the societal needs. The role played by sustainable development policies in earthworks impels to search for alternative construction materials and techniques, to reduce costs, non-renewable energy demand and environmental impact—mainly gas emissions—to a minimum.

Among the possible strategies, biological techniques have received increasing attention in the recent years. They have been exploited in many fields of engineering [10, 21, 55], with a non-exhaustive list including: restoration of rock monuments [28, 46, 50], bioremediation and immobilisation of heavy metals [16–19, 22, 30, 54], shallow carbon sequestration [31, 45], wastewater treatment [24], repair of fissures in concrete [4, 43, 57] and recovery of heavy oil from oil fields [15, 20, 38].

In the field of geotechnical engineering, biological techniques have been mainly assessed on sands [9, 11, 12, 53, 56], where the large pores permit the microorganism life and mobility. Improvement of the hydro-mechanical properties of natural sandy soils has been extensively studied by a number of researchers [1, 9, 11–13, 20, 26, 39, 51, 56]. Most of these studies have been focused on the enhancement of shear strength [27] and on the reduction of soil hydraulic conductivity [48]. Most often described applications include strengthening of foundation [27], settlement reduction [32], soil improvement in retaining walls, embankments and dams [52], increase of resistance against seismic-induced liquefaction [37], and improved oil and gas extraction by reducing sand production and increasing well stability [29].

Information concerning microbiological treatments on compacted soils for earth construction is very limited, despite its potential practical relevance (see for instance [13]), as a valuable alternative to more traditional and more expensive—in terms of both costs and environmental impact—improvement techniques. There are probably two main reasons why microbiological treatment for earth construction has not been considered extensively so far. Firstly, compaction is already considered an improvement technique, and the result of the combination of the two technologies is not easy to foresee. Secondly, the optimal sequence of operations in construction is not straightforward. Indeed, if microorganisms are added before compaction to the soil–water mixture, the soil properties will improve before construction, possibly reducing the compaction efficiency. In turn, the compaction will decrease the effects of the previous biological improvement. On the other hand, if microorganisms are introduced in the soil after compaction, the resulting dominant pore size may be too small for the microorganisms to survive [33, 40, 44].

Notwithstanding potential limitations, a cooperative research between the University of Almería and Acciona Infraestructuras was initiated on microbiological improvement of compacted soils. The latter is the industrial partner in charge of the construction of an extensive highway network in the South of Spain. An experimental study was performed on different soils conventionally used as construction materials, ranging from coarser silty sands to finer silty clayey soils [34]. The first of the two working sequences was chosen by the industrial partner, who decided to add microorganisms before compaction, without any artificial nutrient, to reduce costs and environmental impact. The soft treatment proposed, in which the nutrients required for the cementation process generated by microorganisms are limited to their natural availability, was also intended to keep the potential impact of ammonia and their oxidised forms (nitrates) produced during this treatment to a minimum. Besides environmental impact, oxidation of ammonia should be also avoided due to their acidifying consequences on dissolution of the precipitated calcium carbonate.

Samples were prepared in the laboratory to reproduce the field operational procedures. A comprehensive laboratory investigation was then performed to assess the hydro-mechanical behaviour of the bio-treated soil. The main relevant properties for earthwork constructions, namely water retention, water permeability, compressibility on loading, volume change on wetting, shear strength and small-strain shear stiffness, were investigated. The study was complemented by mercury intrusion porosimetry and scanning electron microscopy, which helped in providing a comprehensive picture of the consequences of the biological treatment on the natural soil.

Here, the results are presented of the comprehensive set of tests performed on silty clayey sand subjected to the industrial soft microbiological treatment. The laboratory tests provide a coherent picture of the hydro-mechanical behaviour of the bio-treated and compacted natural fine-grained soil. The experiments suggest that some improvement can be achieved by the proposed bio-mediated protocol, in spite of the soft procedure adopted and the difficulty in replicating exactly the quantitative results.

2 Background of microbiological soil improvement

Various bio-mediated processes have been studied recently to improve soil performance, including bio-clogging, bio-polymers formation, biogas generation and bio-cementation (see DeJong et al. [6] for an extensive overview). Microbial-induced calcite precipitation is by far the most studied technology to improve stiffness and strength of soils. In brief, the technique relies on the potential of some microorganisms for catalysing calcite precipitation under favourable bio-chemical conditions [14]. Most of the previous research concentrated on calcite precipitation by means of ureolytic bacteria [7, 8, 49], which are able to produce the urease enzyme. The latter catalyses the hydrolysis of urea $CO(NH_2)_2$ to ${CO_3}^{2-}$ and ammonium cation, resulting in an increase in pH and in carbonate concentration in the bacterial environment [49]:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

In the presence of calcium ions, the solution can become supersaturated with respect to calcium carbonate leading to its precipitation:

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \quad (2)$$

Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on the bacterial cell wall once supersaturation is achieved. Contrarily to other methodologies, production of carbonate by hydrolysis of urea can be easily controlled [25]. Recent studies indicate that the concentrations of calcium and nutrients influence the amount and type of precipitates that are formed [1, 10, 28, 35, 42], hence the efficiency of the bio-deposition process. By properly controlling the bio-chemical conditions, high concentrations of carbonate within a short time period can be produced. While enzymatic hydrolysis of urea is the most efficient process in calcite precipitation, other processes, such as sulphate or iron reduction, are also reported to contribute to some extent to the total calcium carbonate precipitation [6].

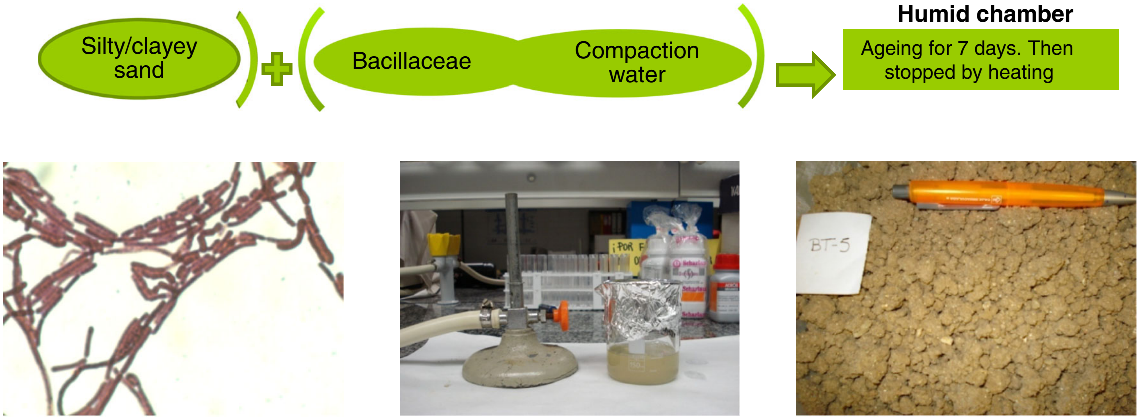
3 Soil properties and microbiological protocol adopted in this study

In this investigation, a soft treatment was chosen by the industrial partner, which consisted in inoculating bacteria to a superficial soil, without adding neither calcium nor nutrients, but relying only on the natural availability of urea and Ca^{2+} in the soil. Urea is found in the natural organic matter content in the soil, while the different sources contributing to Ca^{2+} supply are exchangeable calcium content in the soil, gypsum dissolution at high pH of the soil environment and calcium availability in the mixing water.

Various soils used in construction in the South of Spain were preliminary tested (see Morales [3]), but the laboratory investigation discussed here mostly concentrated on one of them. The way in which the industrial partner decided to operate does not allow for controlling the amount of calcite produced, and results in a non-perfectly repeatable protocol, as the available calcium and nutrients depend on the soil and on the compaction water. However, a coherent pattern of behaviour emerges from the results of the laboratory tests, as will be shown hereafter. Chemical analyses were performed on the natural and the bio-treated soil, as an aid in the interpretation of the results of laboratory tests.

Microorganisms of the Bacillaceae family were added to the compaction water content, as previously reported by Morales et al. [41], without sterilising the soil prior to inoculation. The mixing water had a pH = 7.32, an electrical conductivity of 1.27 mS/cm, 310 mg/L of $CaCO_3$ and 51 mg/L of $MgCO_3$. The soil was mixed with the prepared water to reach water contents between $w = 0.13$ and 0.15, and it was allowed ageing under a relative humidity higher than 97 % for a minimum of 7 days. The bacterial activity was then stopped by increasing the temperature, so that no further bacterial activity interfered with the following hydro-mechanical tests. During the ageing period after the initial mixing, the samples underwent some drying despite being stored under high controlled relative humidity. A second batch of water with the same chemical properties was added to compensate for this small water reduction and restore the water content to $w = 0.15$. Figure 1 summarises the steps followed in the biological treatment before compaction.

The soil is silty clayey sand from SE Spain, widely used in earthwork constructions. It was retrieved from the top layers in situ, which justifies its organic matter content. High-intensity peaks of quartz, albite, chlorite and microcline were identified by random orientation of the powdered material in X-ray diffraction XRD analysis of crystalline phases. The XRD analysis did not detect



(a) Microorganism of the Bacillaceae family (size > 1μm)

(b) Microorganisms added to compaction water

(c) Aggregated soil after ageing

Fig. 1 Steps followed on microbiological treatment before compaction

Table 1 Properties of untreated and treated samples

Sample	Liquid limit	Plasticity index	Particle size			Solid density	Specific surface
	w_L	*PI*	% Finer			ρ_s	S_s
	(%)	(%)	4.75 mm	425 μm	75 μm	(Mg/m^3)	(m^2/g)
Untreated (crushed aggregations)	48	30	100	70	48	2.73	33
Treated	49	29	40	2	0	2.74	32

appreciable calcite, possibly because of its low mass fraction and shielding of other dominant phases. The relevant geotechnical classification properties of the natural soil and of the treated sample are reported in Table 1. The data show that neither the liquid limit nor the specific surface obtained by nitrogen adsorption technique changed to a large extent during the treatment, while adding microorganisms to the soil promoted the formation of aggregates as indicated by the values of the finer fraction.

The apparent grain size distribution, obtained by sieve and hydrometer analyses following ASTM Standards, is shown in Fig. 2, where the particle size distribution of the original soil with natural aggregations is compared to that of the natural powder and to that of the soil after treatment. The comparison of the particle size density functions, plotted in the same figure, highlights that the microbiological treatment tends to aggregate the soil, so that eventually an apparent coarser distribution results. The grain size distribution of the untreated soil with natural aggregations is characterised by a dominant particle size around 10 mm, whereas a dominant value of 20 mm is detected in the coarser-treated material. On water addition (with microorganisms), soil particles tend to aggregate together. The subsequent small drying during the ageing period induces some additional stiffening of the artificial aggregates (shrinking of aggregates), while the biocementation process develops. Figure 3 shows these aggregations after treatment, which display an almost spherical shape with rounded surface.

Table 2 summarises the chemical parameters obtained for the untreated and the treated soil, which reflect the activity of bacteria during soil mixing and ageing, including information on the soluble salt content, electrical conductivity and the total cation exchange capacity with particular emphasis on the exchangeable calcium capacity. The soil maintains an alkaline pH as a consequence of the urease activity, while the organic matter, acting as a carbon (urea) source, decreases (a reduction of 0.23 % corresponds to a demand of 3.9 kg of organic matter per m^3 of compacted soil). The percentage of gypsum after treatment also decreases (reduction of 0.79 %), as a consequence of its dissolution at the high pH of the soil environment, while sulphate content increases in the treated soil. Dissolution of the gypsum is a source of Ca^{2+} supply (3.1 kg of available calcium per m^3 of compacted soil). Availability of about 3.0 kg of calcium per m^3 of compacted soil can be also estimated by considering the reduction in exchangeable calcium capacity. A gross estimation of the different contributions of calcium (exchangeable cation, gypsum dissolution and calcium in the mixing water) is 6.2 kg per m^3

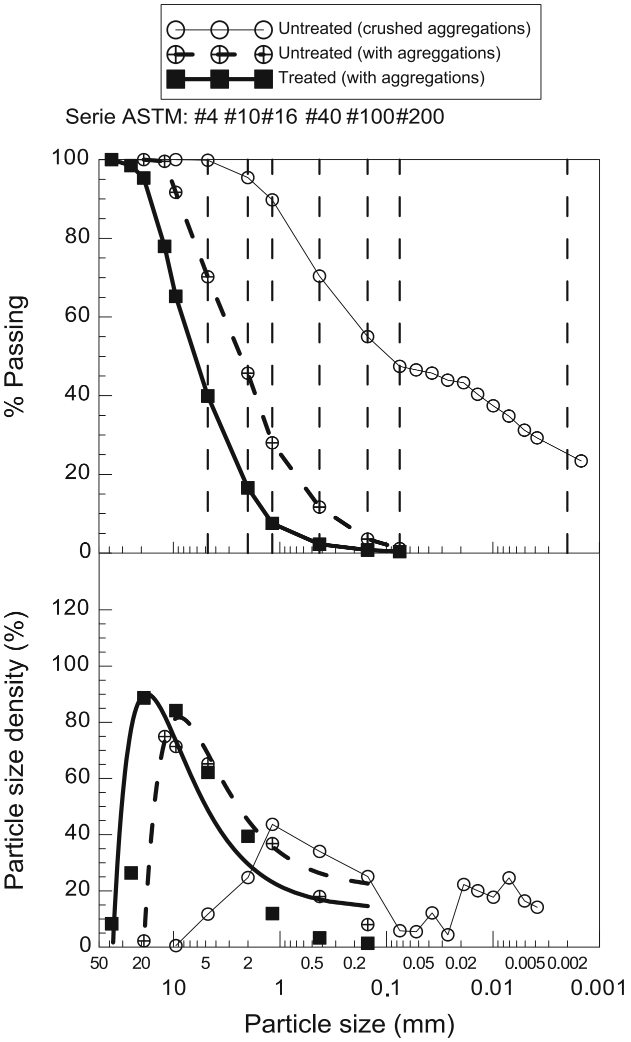


Fig. 2 *Top* Particle size distributions of untreated and treated soils. *Bottom* Particle size density function of untreated and treated soils

Fig. 3 Aggregates formed during microbiological treatment

Table 2 Chemical properties of untreated and treated samples

Sample	pH	Organic matter	Gypsum	Sulphates	$CaCO_3$	Soluble salts	Electrical conductivity	Cation Exchange Capacity	
		(%)	(%)	(mg/L)	(%)	(%)	(dS/m)	(meq/100 g soil)	Ca^{2+} (cmol/kg)
Untreated	9.4	0.39	0.91	11.30	1.1^a–4.5^b	0.04	0.54	17.80	23.82
Treated	8.9	0.16	0.12	231.3	1.8^a–6.4^b	0.08	1.27	22.30	19.34

[a] Bernard

[b] Dietrich-Frühling

of compacted soil. Calcium carbonate content increases between 0.7 % (Bernard method) and 1.9 % (Dietrich-Frühling method). A calcium carbonate increase of 0.7 % (around 12 kg per m^3 of compacted soil) requires 4.7 kg of available calcium per m^3 of compacted soil.

In Fig. 4, the dynamic compaction curves of the untreated natural material compacted at four different energy levels are compared with each other: 1/2 Standard Proctor (1/2 SP in the figure at 0.3 MJ/m^3), Standard Proctor (SP at 0.6 MJ/m^3), 2 Standard Proctor (2 SP at 1.2 MJ/m^3) and Modified Proctor (MP at 2.7 MJ/m^3). Standard Proctor compaction performed on the untreated soil indicated an optimum dry density of 1.85 Mg/m^3 (top of shaded area) at a water content of $w = 0.15$ (void ratio $e = 0.47$ and degree of saturation $S_r = 0.87$). Most of the untreated soil samples used in the test programme were prepared by Standard Proctor compaction, on the dry side of optimum, at water content between w_{opt} and ($w_{opt} - 2$ %) as indicated by the shaded area in Fig. 4. These compaction conditions are assumed to be representative of field conditions in the arid climate of SE Spain. Following the most likely field practice, the treated soil was also dynamically compacted after ageing by Standard Proctor compaction at the water content $w = 0.15$. The reason for choosing the same compaction conditions was to isolate the effects of the bio-mediated treatment from those of the compaction energy.

The aggregates formed during mixing and ageing, reported in Fig. 3, limit the efficiency of compaction energy in reducing the void ratio of the soil. Part of the compaction work input per unit soil volume is dissipated in breaking the coarser aggregates and the organogenic bonds. The compacted treated samples have a higher initial void ratio compared to that obtained for the untreated soil, as can be observed in Fig. 2. A dry density of 1.72 Mg/m^3 is reached at $w = 0.15$ ($e = 0.59$ and $S_r = 0.70$) corresponding to the bottom of the shaded area in the figure, which approximately coincides with the density obtained using 1/2 Standard Proctor energy on the untreated material.

For the sake of comparison, few treated samples were statically re-compacted after Proctor compaction, to the same void ratio of the untreated material (samples further identified as 'treated_RC'). In this way, some of the effects, which could be attributed to different initial void ratios, could be better analysed.

4 Preliminary evaluation of ageing effects from small-strain stiffness

A preliminary set of samples was prepared in a slightly different way, to evaluate the improvement by calcite precipitation under natural availability of calcium ions

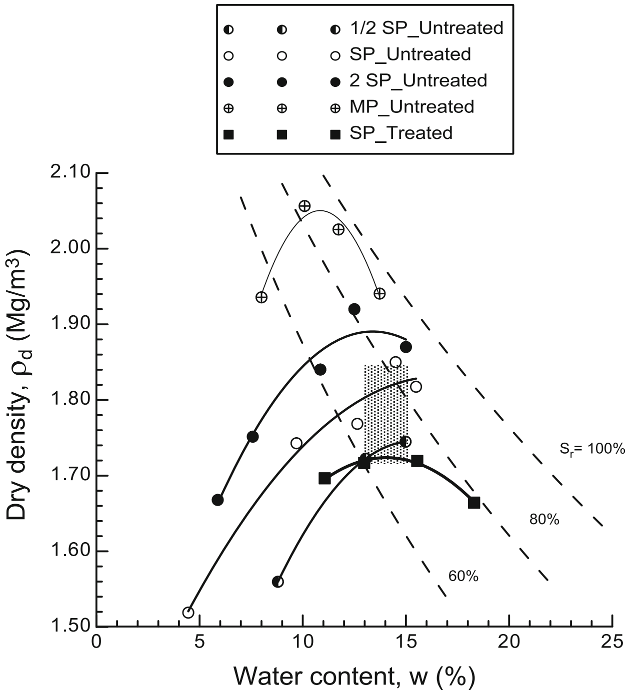


Fig. 4 Dynamic compaction *curves* at different energies for treated and untreated soils. The *shaded area* represents the as-compacted conditions used in the test programme

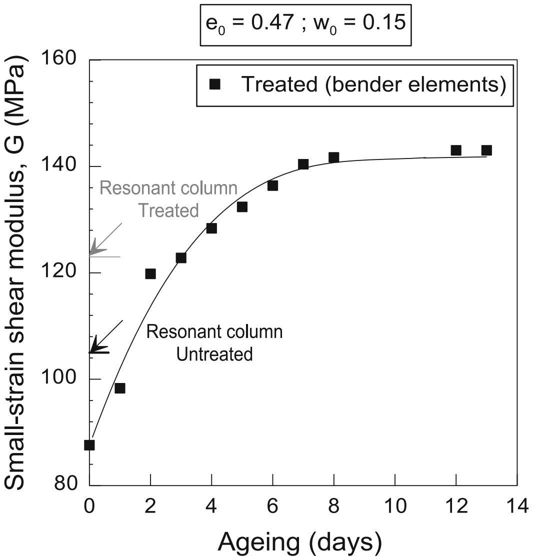


Fig. 5 Time evolution of the small-strain shear stiffness increases (bender elements under unstressed conditions) during ageing of a previously compacted soil. No water content losses were allowed during ageing. Resonant column test results on untreated and treated samples under a confining stress $\sigma_c = 0.1$ MPa (treated samples were statically compacted after 7 days ageing)

and nutrients, on the soil during ageing. The samples were statically compacted to the target void ratio at the reference compaction water content, $e = 0.47$ at $w = 0.15$, corresponding to Standard Proctor of the untreated material. The compacted samples were left for a maximum of 13 days inside a desiccator at controlled high relative humidity and room temperature, and the evolution of small-strain stiffness was periodically evaluated during ageing. In this case, the biological activity was taking place after compaction.

Under a relative humidity higher than 97 %, the samples showed an initial total suction of about 1 MPa. Two relevant mechanisms are expected to take place while the microbiological activity develops on the already compacted soil. On the one hand, precipitation of $CaCO_3$ minerals (Eq. 2) is expected to increase the solid mass during ageing. On the other hand, a reduction in water mass could take place due to the microbiological reactions (refer to Eq. 1). Indeed, the relative humidity was kept constant in the desiccator, in order to isolate the effects of $CaCO_3$ precipitation. In this way, ageing will occur under approximately constant water content, and the water mass used by microbiological reactions will be almost compensated for by available water vapour.

The small-strain stiffness was evaluated using bender elements under unstressed conditions, by continuously monitoring small-strain shear modulus G_0 through the measurements of the shear wave velocity V_S. The shear modulus is calculated as $G_0 = \rho_t V_S^2$, where ρ_t is the total density, and $V_S = l_{eff}/t_S$. The wave travel distance l_{eff} was taken as the distance between the transducer tips. The arrival time t_S was determined by inspection of the received trace, looking for the first significant amplitude change. A peak–peak sine pulse of 20 V with apparent input frequency 15 kHz was used as input signal. Additional details are given in [23, 36].

Small-strain stiffness increases in time, as shown in Fig. 5, but levels off after about 6 days. Similar results were recently obtained by Morales et al. [5] on treated samples, which had been prepared at different initial water contents. Results of torsional resonant column tests under a confining stress $\sigma_c = 0.1$ MPa are added in the figure for the sake of comparison. Maximum small-strain shear stiffness (shear strains below 0.001 %) is reported for a natural untreated sample, and for a treated sample tested after 7 days ageing. A relatively good agreement is observed between the small-strain stiffness results obtained by the different techniques. The differences can be easily explained if reference is made to the actual testing procedures. The resonant column result for the untreated material is slightly higher compared to the starting point of the bender elements test, which is consistent with the slightly larger confining stress applied. Conversely, the small-strain stiffness of the treated material obtained from the resonant column test is slightly lower compared to the levelling off value of the aged material, as detected by bender elements, which starts introducing the role played by different compaction sequences on the improvement technique. The bender elements tests were performed on samples in which the microbiological treatment was applied to the previously compacted soil at $e = 0.47$, while for the resonant column test the sample was statically compacted at the same void ratio after microbiological treatment. The latter procedure resulted in a less stiff material at the end of the curing time, partially associated with the breakage of organogenic calcite bonds created during ageing.

5 Microstructure investigation

To investigate the consequences of the preparation sequence and to provide a deeper view on the fabric changes induced by the treatment, microstructure features of the untreated and treated soils were studied by scanning electron microscopy (SEM) with chemical identification by energy dispersive X-ray spectroscopy (EDS), as well as by mercury intrusion porosimetry (MIP) tests performed on freeze-dried samples to preserve the pore network.

Photomicrographs were obtained by SEM, after bio-mediated treatment but before compaction. Results presented in Fig. 6 show crystal growth in contact with microorganism (cell membrane), as a result of supersaturation of calcium ions in the alkaline environment [47]. The calcified bacteria act as nucleation points for further carbonate precipitation. The carbonate mineral images obtained by SEM, presented in Fig. 7, were studied by qualitative EDS analyses, which revealed the presence of carbon, oxygen and calcium in such an amount that confirms precipitation of $CaCO_3$ crystals in the microbiologically treated soil. EDS analyses on the untreated soil revealed predominance of silicon and aluminium associated with quartz, albite and microcline. In the case of the treated soil, the calcium detected is associated with $CaCO_3$ polymorphs, mainly the amorphous structures and calcite crystals observed with SEM technique.

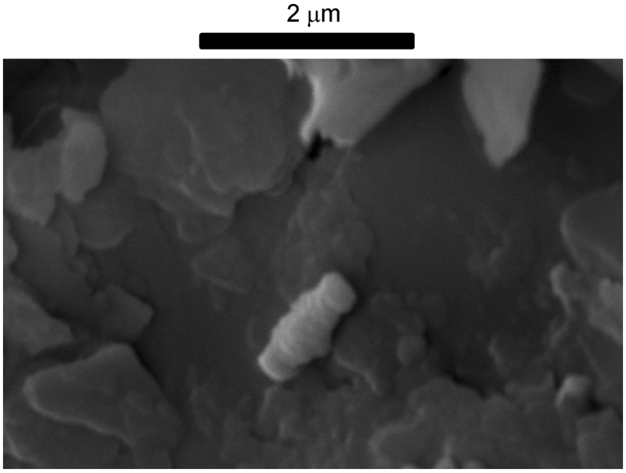


Fig. 6 Photomicrograph obtained by SEM. Calcified bacterium acting as nucleation point

Different precipitation structures can be identified in the figure, namely calcified bacteria (Fig. 7a) and precipitations between soil grains (Fig. 7b). Figure 7b shows carbonate crystals filling large inter-grain pores (bio-filling) and bonding soil grains (bio-cementation).

The cumulative and density distributions of the pore size for the relevant samples, derived from MIP data, are reported in Fig. 8, including the untreated, the treated and the treated and re-compacted samples. The distributions are complemented by nitrogen desorption test, which was obtained by the BJH method [2]. Maximum void ratios determined with the combined methods are slightly lower than the as-compacted void ratio of the untreated material ($e = 0.47$ indicated by a dashed line). The difference arises from the non-intruded porosity for pore sizes smaller than 2 nm and from the non-detectable porosity for pore sizes larger than 400 μm.

The comparison between the three samples allows for highlighting the role played by the compaction sequence. The data reported in the lower panel in Fig. 8 show that the different samples have the same pore size distribution in the range from 3 nm to 3 μm, where a dominant mode around 5 nm is detected by BJH method. The effects of compaction of the bio-treated soil become evident in the range of pores from 3 to 50 μm, bounded by the vertical dashed lines. In this range, the amount of pore space is reduced, which is consistent with the SEM observations reported in Fig. 7b, showing mineral precipitation in the larger pores between the soil grains. The overall result of calcite formation is bio-filling of that pore class, which is compatible with the vital space required by bacteria, with a size around 1 μm (refer to Fig. 6). The dominant pore mode of the untreated material is around 50 μm. A dominant peak around 200 μm is also identified for the treated sample, which is reduced upon further compaction.

6 Hydraulic behaviour: water retention and water permeability

Different techniques were combined to investigate the water retention properties of the natural and the bio-mediated soil, on samples compacted after ageing. Starting from as-compacted conditions at an initial water content $w = 0.15$, both treated and untreated samples were progressively air-dried. A chilled mirror dew point psychrometer (WP4, Decagon Devices) was used to collect data in the range of suction from 1 to 100 MPa. The results plotted in Fig. 9 show that the water retention curves

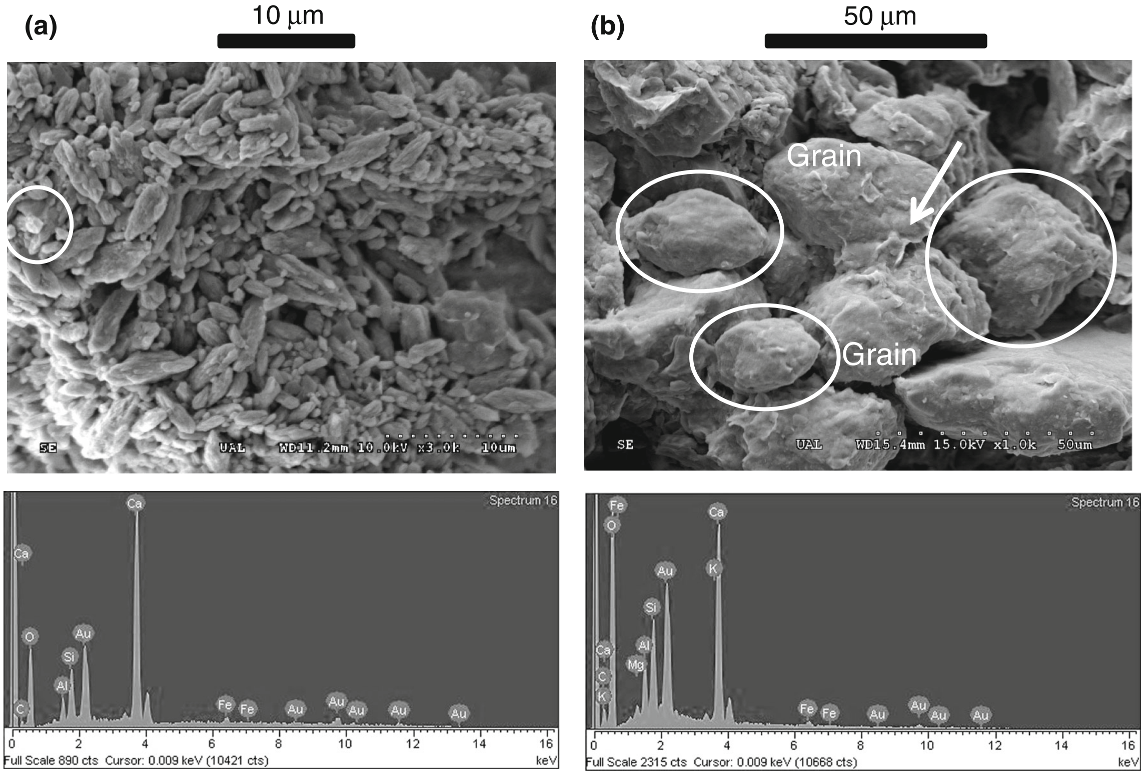


Fig. 7 SEM images and EDS analyses of treated samples before compaction. **a** Photomicrograph with calcified bacteria and calcite crystals and corresponding EDS for the encircled area. **b** Photomicrograph with calcite crystals located between grains of soil. The *arrow* shows organogenic bonds together with their corresponding EDS (at the bottom)

obtained in this suction range for the natural and the treated samples do not differ substantially. In this range, in fact, water retention is mainly governed by adsorption mechanism in the inter-aggregate porosity, which is ruled by the specific surface of the material, as suggested by Romero et al. [47]. Similarity between the two curves in this suction range reflects the fact that the treatment hardly affected the specific surface of the soil (see Table 1).

In the lower suction range, water retention data were collected by means of the axis-translation technique. Starting from an initial water content $w = 0.15$, compacted samples of natural and treated soil were wetted to a matric suction of $s = 20$ kPa under a constant vertical net stress of 50 kPa. After suction equalisation, the samples were dried in steps up to a matric suction of $s = 200$ kPa in the case of the untreated material, and $s = 500$ kPa for the treated one. The experimental data show that the water retention domain of the untreated and the treated soils differ from each other mostly in the suction range from 0.006 to 0.1 MPa, bounded by the dashed lines in the figure. This suction range is associated with the porosity reduction detected by MIP results between entrance pore sizes from 3 to 50 μm. Bio-filling is expected to affect mostly the capillary retention mechanisms in the lower suction range. As shown in the figure, the treated material was able to undergo suction increase up to 500 kPa without significant water content changes, which was not the case for the untreated sample, which started undergoing appreciable water content decrease at a matric suction of about 200 kPa. Further elaboration of MIP data included in Fig. 9 complete the picture of retention behaviour in the very low suction range. A water content decrease is apparent for the treated sample at suctions below 6 kPa, which is the evidence of desaturation of the largest pore mode around 200 μm (refer to Fig. 8) created by bio-mediated aggregation.

Water permeability under saturated conditions was determined on compacted samples of treated and untreated material using an isotropic cell under constant confining stress (100 kPa) and controlled-gradient conditions (a water pressure of 80 kPa was kept constant at the bottom boundary, while the top boundary was maintained under atmospheric conditions). Void ratios were determined after the hydraulic tests by measuring the water content under saturated conditions. Direct flow data were complemented by indirect estimation of water permeability from

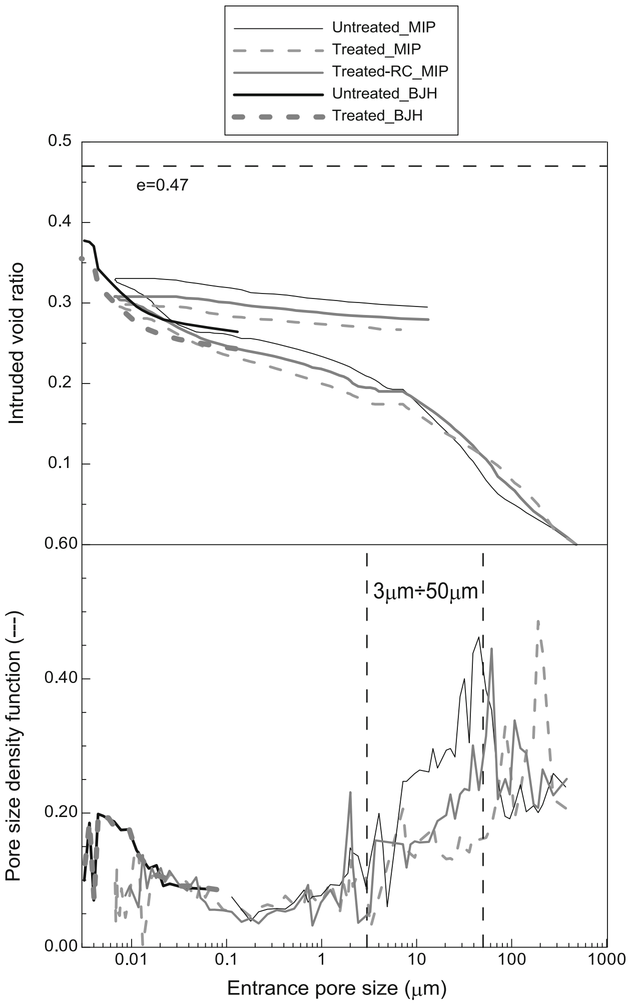


Fig. 8 Cumulative distributions and pore size density functions for untreated, treated and treated/re-compacted samples derived from MIP data

oedometer consolidation tests. The water permeability calculated by means of back-analysis was associated with the average void ratio of the relevant loading steps.

Water permeability obtained with direct measurement (controlled-gradient tests) and by elaboration of oedometer consolidation data is plotted in Fig. 10 for both the treated and the untreated materials as a function of void ratio, together with exponential fits. Time evolution of soil deformation has been interpreted using a nonlinear curve-fitting algorithm to determine the different conventional parameters used in consolidation analysis following Terzaghi's theory. For the same void ratio, the treated samples display consistent lower water permeability than that of the untreated one. This confirms that, once the largest pores are closed under load, the treatment results in bio-filling of that class of intermediate pores highlighted by MIP data, which reduce the overall hydraulic conductivity of the bio-treated samples.

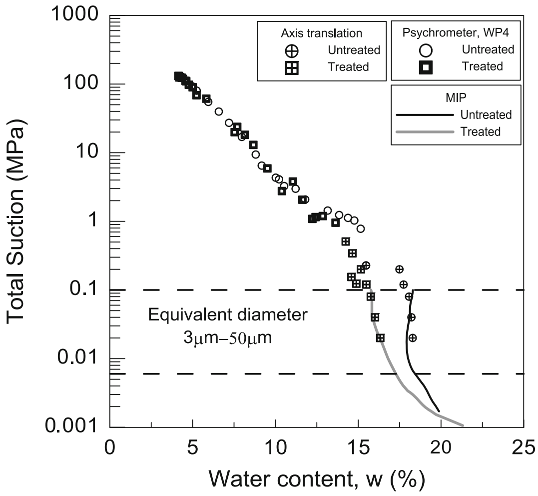


Fig. 9 Water retention curves on drying using psychrometer and axis-translation data for untreated and treated soils. Retention curves in the low suction range estimated from MIP data

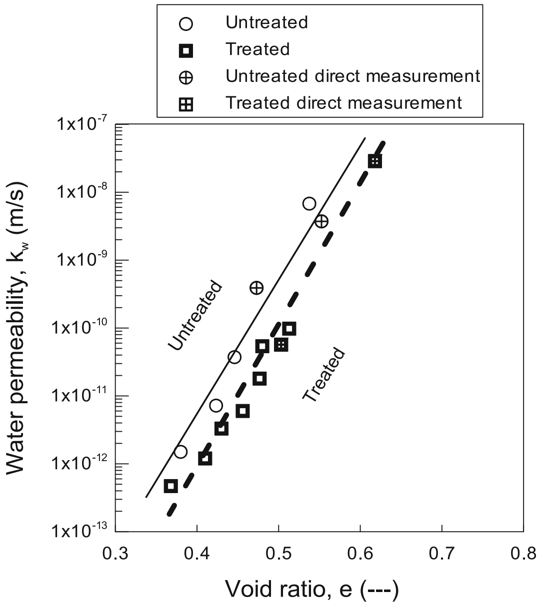


Fig. 10 Water permeability under saturated conditions as a function of void ratio for untreated and treated soils. Direct tests under controlled-gradient conditions in isotropic cell

7 Mechanical behaviour: shear strength, compressibility on loading, volume change on wetting and small-strain stiffness

Direct shear tests were performed on untreated and treated samples, which were compacted after bio-treatment, to analyse the effects of the treatment on the shear strength behaviour of the soil. The tests were performed at water contents corresponding to saturation, to mimic saturation in the field due to water infiltration. To ensure saturation, the as-compacted samples (60 mm in diameter and 20 mm high) were soaked for 24 h under the same vertical stress at which shear was performed. The initial void ratios were around $e = 0.47$ for the untreated soil and slightly higher (around $e = 0.59$) for the treated one. The shearing stage was performed at a controlled displacement rate of 0.005 mm/min, which ensured drained conditions.

Figure 11 shows mobilisation of shear stress and vertical displacement (negative values correspond to compression) during shearing at different effective vertical stresses (50, 100 and 150 kPa). All samples contracted on shearing. The treated soil displays slightly higher shear strength values, which are reflected in the linear shear strength envelopes depicted in Fig. 12, which give a friction angle of about $\phi' = 38°$ for the untreated soil, and of $\phi' = 40°$ for the treated one.

It is worthwhile remarking that the bio-mediated treatment slightly increased the shear strength of the soil, but the shear strength envelope did not show intercept cohesion. Instead, it increased the friction angle, in spite of the slightly higher void ratio of the treated material. Again, the bio-mediated treatment applied before compaction does not result in a bio-cementation effect. On the contrary, the final effect on the shear strength is somehow equivalent to that produced by an increase in the relative density of a granular soil. This evidence is consistent with the idea that the intermediate pore class between 3 and 50 µm, corresponding to inter-grain or inter-aggregate porosity, is partially filled with crumbled calcite, which eventually increases the density of the aggregated soil.

To study the stabilisation effect of bacteria on the compressibility of the soil, oedometer tests were run on the untreated soil and on the soil compacted after bio-treatment. Some tests were also run on the treated soil that was further statically compacted to reach the void ratio of the compacted untreated material (treated_RC). All tests were conducted following the same protocol. After mounting the samples at the initial water content ($w = 0.15$) in the oedometer cell, the vertical stress was increased in steps until a value of $\sigma_v = 50$ kPa or $\sigma_v = 100$ kPa. Once the desired stress level was reached, the samples were soaked at constant vertical stress for 24 h. Afterwards, the saturated samples were loaded in steps of 24 h each up to 1 MPa or 2 MPa, depending on the test. Finally, the samples were unloaded, again in steps of 24 h each. The data of the tests in which the samples were flooded at $\sigma_v = 50$ kPa are shown in Fig. 13. All the samples showed volumetric collapse upon wetting. The treatment apparently stiffens the soil in the post-yield domain, as the comparison of the compression curves after volumetric collapse suggests.

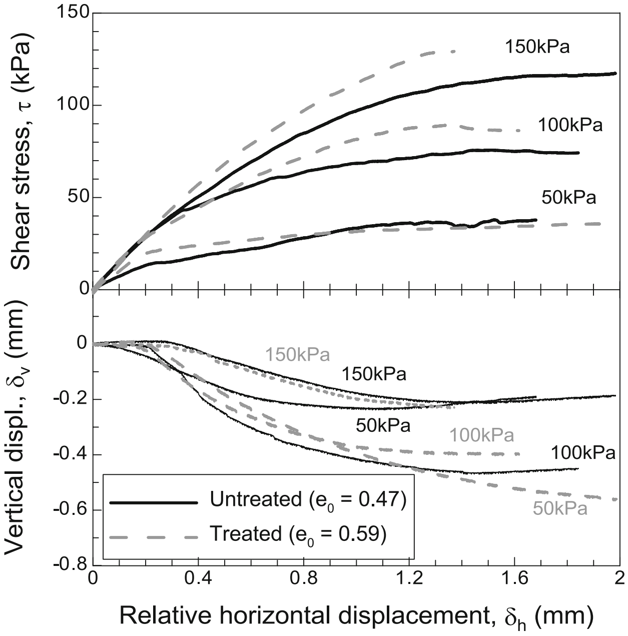


Fig. 11 Direct shear data for untreated and treated soils (0.005 mm/min of displacement rate): **a** shear stress evolution, and **b** evolution of vertical displacement during shearing

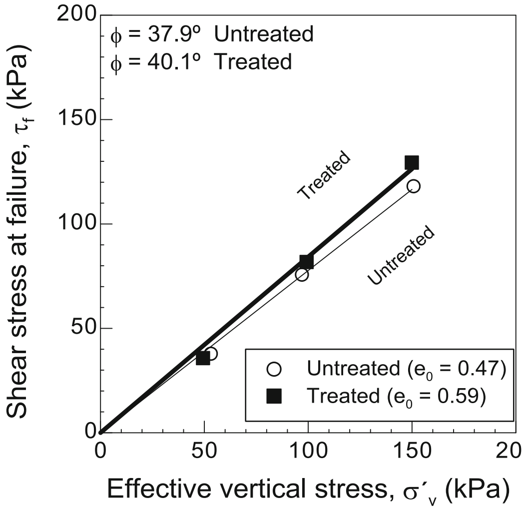


Fig. 12 Linear shear strength envelopes for untreated and treated soils

Compression curves for the samples flooded at $\sigma_v \geq 100$ kPa are shown in Fig. 14. The data show that the as-compacted void ratio initially dominates the amount of collapse. The highest reduction in void ratio is experienced by the untreated sample, which is the one having the highest void ratio at the beginning of wetting, while the re-compacted samples displayed almost no collapse upon wetting consistently with its lower initial void ratio.

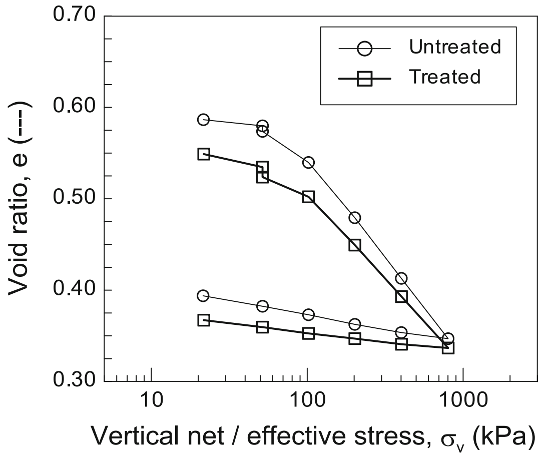


Fig. 13 Oedometer compression *curves* for untreated and treated soils. Samples flooded at 50 kPa

In order to evaluate durability of the bio-mediated treatment under aggressive chemical conditions, two additional oedometer tests were run on samples, which were chemically attacked during soaking after treatment. For these samples, identified as 'treated decalcified' in the figure, acetic acid at initial pH = 3 was used during the soaking stage. The soaking stages were performed at $\sigma_v = 100$ and 200 kPa, respectively, for the two samples analysed. As observed in the figure, despite starting at approximately the same initial void ratio, the decalcified samples displayed a larger collapse compared to the sample which did not undergo chemical attack, which is due to dissolution of bio-mediated calcite.

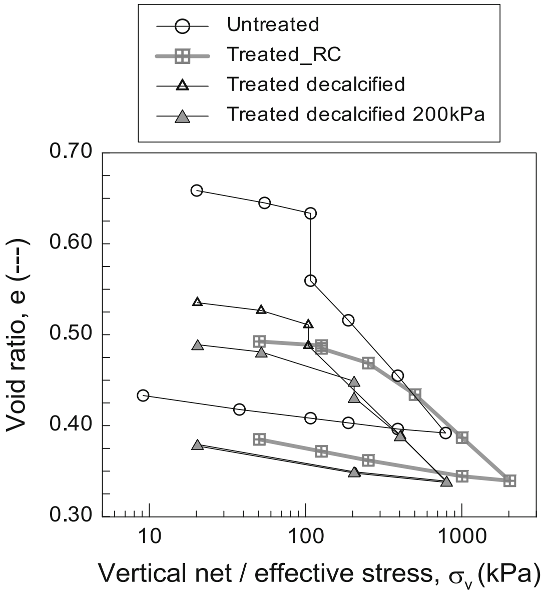


Fig. 14 Oedometer compression curves for untreated and treated soils. Samples flooded at 100 and 200 kPa

The amount of collapse undergone upon flooding by the different samples was normalised with the vertical net stress at the time of wetting. The data reported in Fig. 15 as a function of the void ratio at the time of flooding confirm that the amount of normalised collapse experienced by the natural and the treated soils is ruled primarily by the void ratio. Nevertheless, consistent reduction of collapse is observed for treated samples at comparable void ratios with respect to the natural soil. Chemical attack partly hinders the effects of the bio-mediated treatment, although, in any case, the collapse potential is reduced also in this case, at comparable void ratio.

The total collapse potential can be considered to be given by the sum of the collapse potential of the largest pores, which are hardly affected by bio-mediated treatment, and of the pores in the intermediate range (sizes between 3 and 50 μm), which are filled by calcite formation. As the largest pores are held responsible of most of the volumetric collapse upon wetting, the initial void ratio plays the most relevant role in the amount of collapse. Nonetheless, at comparable void ratios, the effect of bio-filling is to further reduce the amount of collapse upon wetting.

Figure 16 summarises the compressibility parameters on loading for the untreated, treated, treated and re-compacted, as well as treated and decalcified samples. Both elastic κ_{oed} and elastic plastic λ_{oed} one-dimensional compressibility under saturated conditions were analysed. The compressibility parameters were calculated as $-\Delta e/\Delta \ln \sigma'_v$, where σ'_v is the vertical effective stress, and they are plotted against the void ratio at the end of the tests. The untreated soil has a compressibility around $\lambda_{\text{oed}} = 0.089$, and the slope of its unloading–reloading line is in the range $\kappa_{\text{oed}} = 0.009$–$0.013$. Treating the soil with bacteria reduces the elastic plastic compression slope to $\lambda_{\text{oed}} = 0.080$ and the elastic compressibility to values around $\kappa_{\text{oed}} = 0.009$.

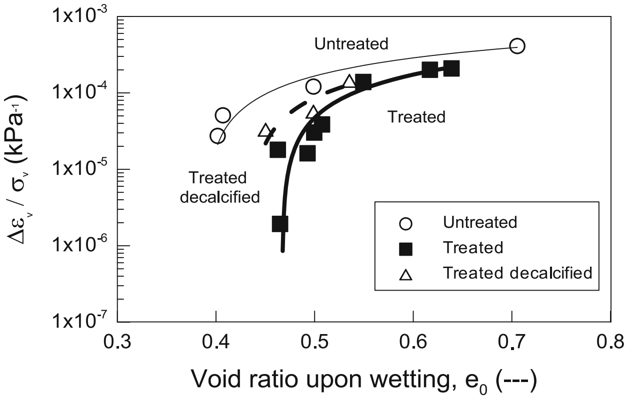


Fig. 15 Collapse strain during soaking—normalised with vertical net stress—versus void ratio upon wetting

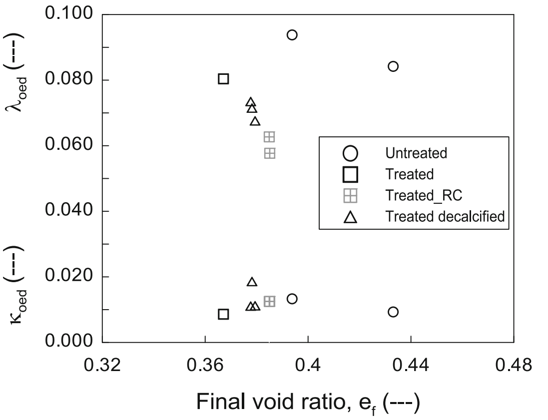


Fig. 16 Elastic κ_{oed} and elastic plastic λ_{oed} compressibility parameters under oedometer conditions for untreated, treated and decalcified samples, as a function of void ratio at the end of the tests

Re-compacting the treated soil to smaller void ratios, further decreases the slope of the elastic plastic compression line to values around $\lambda_{oed} = 0.061$, while it does not have any significant further effect on the slope of the unloading–reloading line. Decalcifying the treated material does not imply recovering the total compressibility of the natural, untreated soil. The average compressibility of the decalcified samples, $\lambda_{oed} = 0.071$, is in between those of the natural and the treated samples, and closer to the latter.

Investigation on the influence of the bio-mediated treatment on the mechanical properties of the soil was completed by analysing small-strain shear stiffness at different net confining stresses in resonant column tests. The Stokoe resonant column used is a fixed-free type under torsional mode of vibration, in which the soil specimen is fixed at the bottom platen and free to oscillate at the top cap. An accelerometer fixed to the drive plate is used to determine the shear strain amplitude at the first mode of resonance of the specimen. The small-strain shear stiffness as a function of shear strain at two confining net mean stresses (0.1 and 0.3 MPa) is presented in Figure 17 for both the natural and the treated material (after 7 days ageing). In this case, static compaction was used to bring untreated and treated samples exactly to the same initial condition ($e = 0.47$ and $w = 0.15$ corresponding to Standard Proctor of the untreated material). Treated samples consistently displayed higher small-strain shear stiffness in the shear strain range investigated, despite the fact that static compaction after ageing partly destroyed the organogenic bonds promoted by bacterial activity, which were claimed to be responsible for the small-strain stiffness increase presented in Fig. 5.

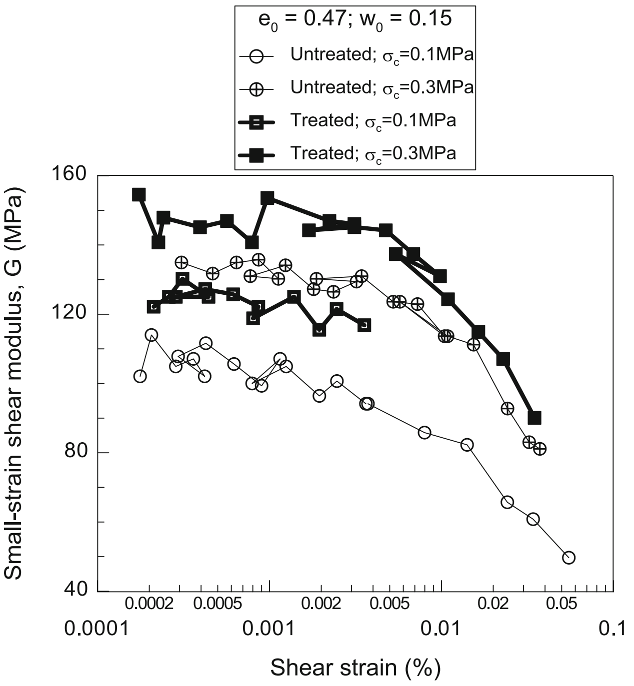


Fig. 17 Small-strain shear moduli obtained by resonant column tests at different confining stresses on untreated and treated soils. Treated samples (after 7 days ageing) were brought to the same initial condition ($e = 0.47$ and $w = 0.15$)

8 Summary and concluding remarks

An experimental investigation has been undertaken at the University of Almería (Spain) to analyse the possibility of stabilising compacted soils by a soft bio-mediated treatment. The treatment proposed by the industrial partner consists in adding microorganism to the compaction water content and relying on the natural availability of urea and Ca^{+2} in the superficial soils used in the construction. Such a choice was motivated by the necessity of reducing the cost of the technique and its environmental impact with respect to alternative solutions.

Here, the hydro-mechanical effects of the treatment were analysed on a silty/clayey sand, extensively used in earthwork constructions, by means of a comprehensive series of laboratory tests and techniques, including an analysis of the treated soil fabric, its hydraulic and mechanical properties.

A preliminary overview of the consequences of the treatment was provided by the evolution of small-strain shear stiffness tracked by bender elements during the incubation process that lasted 13 days. Samples were compacted and then bio-treated, and the increase in stiffness during ageing was associated with organogenic bonds created during carbonate precipitation. Bio-cementation phenomena were also detected in SEM images and EDS analyses, which confirmed the formation of $CaCO_3$ crystals filling large inter-grain pores and bonding soil grains.

The construction protocol suggested for field applications consists in compaction after ageing, with microorganism added to the water content prior to compaction. Most of the samples tested in the laboratory were prepared by Standard Proctor compaction at the optimum water content for the untreated soil, $w = 0.15$, which replicated the expected field practice.

Such a procedure limits the efficiency of the biological treatment, and it is not expected to be completely reproducible quantitatively, as the amount of nutrients and availability of calcium ions will depend on the natural soil and the compaction water, in general. Nonetheless, the whole set of laboratory tests provide a coherent picture of the potentialities and limitations of the proposed technique.

Adding microorganism to the soil promotes the formation of an aggregated structure, which remains after compaction. Precipitation of calcite from bacteria takes place in the pores of the soil, which are slightly larger than the characteristic size of the bacteria, which is around 1–2 μm. The following compaction breaks the coarser aggregates and reduces the bio-cementation effect, which was initially detected by bender elements and microstructure analysis. As a final result, the precipitated crystals tend to fill the pores in the range 3–50 μm, and the pore volume of the material tends to decrease as a consequence of the progressive filling of the inter-grain/inter-aggregate porosity.

The change in the pore size density function is reflected consistently in the hydraulic and mechanical behaviour of the treated soil, which presents typical features of a denser soil with respect to the untreated one. Filling part of the soil macroporosity of the treated samples affects the water retention properties inducing slightly higher air-entry value and lower water permeability at comparable void ratios. Treated soils display a slightly higher friction angle with no cohesion in the shear envelope, consistently with the pattern of a denser granular soil. The small-strain shear stiffness increased, and collapse on wetting for a given initial void ratio reduced together with the post-yield compressibility on loading.

The body of experimental data collected suggests that the proposed microbiological technique may be effective in improving a little the hydro-mechanical characteristics of the compacted soil. The advantage of the proposed *soft* technique relies mostly on its low cost and its low environmental impact, compared to possible alternatives. Biocementation is not fully exploited, but the resulting change in pore size distribution is sufficient in increasing the performance of the construction material. Nonetheless, due to the aggregated structure and the bio-mediated bonds initially created during ageing, best practice will require more compaction energy to reduce the total void ratio.

As the treatment is not completely controlled in terms of bio-chemical conditions, heterogeneity will result in the practice, which will require a statistical analysis of the compacted soil properties if the treatment is chosen effectively in field construction.

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