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Effects of inoculation with *Bacillus arenosi* on physical and biochemical properties of soil, and surface runoff and erosion in degraded forestlands of Northern Iran

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Abstract

A viable practice to improve key properties of soil, and reduce runoff generation and erosion may be inoculation with selected bacteria. However, no previous studies have evaluated whether the direct inoculation of *Bacillus arenosi* K64 (hereafter simply indicated as *B. arenosi*) to soil impacts the main physical and biochemical properties of soil as well as the runoff and erosion rates. This study has measured the infiltration rate (IR), tensile strength (TS), mean weight diameter (MWD), polysaccharides (PS) and important enzymes of soil (β -glucosidase, N-acetyl glucosaminidase, phosphatase and arylsulphatase) in degraded forest soils (Northern Iran) inoculated with *B. arenosi* in comparison to untreated soils (control). Moreover, the runoff coefficient and soil loss have been measured in plots with soil sampled in those sites under natural rainfalls. IR, TS and MWD increased by 65% to 160% in treated soils compared to the control. PS and enzymes were significantly higher (by 20% to 200%) after soil inoculation. A noticeable decrease in both surface runoff and soil loss (both by 45%) in the inoculated soils resulted from those changes. High correlations between the hydrological response of soil on one side, and the measured physical and biochemical variables on the other side were observed. A derivative variable calculated by the Principal Component

Analysis can be considered as a meaningful parameter to estimate the changes in soil properties and response due to inoculation with bacteria. Agglomerative Hierarchical Cluster Analysis clearly discriminated the treated from the untreated soils. This study has demonstrated that soil inoculation with *B. arenosi* plays a significant role in reducing runoff generation and soil erosion under the experimental conditions, thanks to the general improvement in physical and biochemical properties of soil.

Keywords: water infiltration; soil aggregate stability; polysaccharides; soil enzymes; runoff generation; soil loss.

1. Introduction

Soil erosion negatively impacts the environment throughout the world (Rodrigo-Comino et al., 2018). Erosion is of particular concern in forest ecosystems, where unsuitable soil management practices and other anthropogenic actions (such as deforestation, logging, fraudulent fire) increase erosion rates (Bozali, 2020; Parhizkar et al., 2023c). The use of bio-based techniques for ecosystem restoration, especially targeted to soil quality improvement, may be cheap and environmentally sound solutions to reduce the negative impacts of soil erosion (Kheirfam et al., 2020).

The scientific literature reports many studies that have assessed the effectiveness of different soil amendments at reducing soil detachment rates, such as sawdust ash (Medina-Martinez et al., 2023), lime (Banu and Attom, 2023), organic compounds (Ding et al., 2021), plant residues (Wilson et al., 2008), biodegradable plastic (Abbate et al., 2023), gypsum (Kushwaha and Kumar, 2023) and biological agents (Mahmud et al., 2021). Improving soil properties by using native biological amendments has been shown to be a suitable practice to control the hydrological and erosive response of soil (Sadeghi et al., 2017). This practice avoids economic and environmental issues linked to applicability, cost and durability of chemical additives for soil conservation (Jafarpour et al., 2022). Many studies have demonstrated that, among the biological agents,

microorganisms, such as bacteria and fungi, can improve soil properties (e.g., Kheirfam et al., 2017; Muñoz-Rojas et al., 2018) and therefore reduce runoff yield and soil erosion (e.g., Sadeghi et al., 2017; Kheirfam et al., 2020). The soil microorganisms secrete exopolysaccharides and mucopolysaccharides, supporting formation of soil macro-aggregates and increasing porosity (Zhang et al., 2021b), roughness (Rodríguez-Caballero et al., 2012), stability (Degens et al., 1997), and organic matter content (Kheirfam et al., 2017) in treated soils. These effects directly increase soil infiltration and water retention (Chamizo et al., 2012). For example, Sadeghi et al. (2017) showed that *Bacillus subtilis* is able to increase infiltration rate, one of the most important hydraulic parameters controlling soil hydrology. Changes in water infiltration rates due to the effects of bacteria inoculation generally result in decreases in runoff coefficient (Kheirfam et al., 2020; Jafarpour et al., 2022) and soil loss (Kheirfam et al., 2017; Sadeghi et al., 2020). As an example, Sadeghi et al. (2021) demonstrated that bacterial inoculum decreased soil loss by three to seven times compared to untreated soils. Kheirfam et al. (2017) stated that soil bacteria can considerably reduce soil erosion by increasing soil stability. This means that bacteria are particularly useful for soil conservation in degraded forestlands that are susceptible to soil erosion once they lose vegetation cover and diversity (Parhizkar et al., 2020a; 2023b; Shabanpour et al., 2020). However, in spite of the aforementioned studies, the high diversity of soil biota makes the effectiveness of some microorganisms at improving soil properties and specifically water infiltration non-unanimous in the scientific community and among land managers. It is worth mentioning that, among the various genera of soil bacteria, few of these microorganisms are used in the inoculation process for improving soil conditions. For instance, to the authors' best knowledge, no previous studies have evaluated how much direct inoculation of *Bacillus arenosi* K64 (hereafter *B. arenosi*) into soil impacts its hydraulic properties, and consequently surface runoff and soil erosion. Moreover, the associations between the changes in soil properties (e.g., water infiltration rate, soil

aggregate stability, and tensile strength) and its hydrological and erosive response to rainfall on one side, and some important soil enzymatic activities after inoculation with bacteria have not been explored as far as now. These associations are expected, considering the aforementioned effects of soil microorganisms on key properties of soil. Therefore, their identification is essential, in order to better understand how a microbial strain influences runoff generation and particle detachment compared to untreated soils. To fill this gap, this study evaluated the effects of soil inoculation of *B. arenosi* on some important physical and biochemical properties of soil as well as on surface runoff and soil loss compared to untreated soils. A case study was identified in a forest park of Northern Iran, where the impacts of soil erosion are severe. The specific objectives of the study are, by comparing inoculated and untreated soils: (1) to evaluate the infiltration rate, tensile strength and aggregate stability of soils; (2) the analysis of extracellular polysaccharides and enzymatic activities; and (3) the estimation of the runoff coefficient and erosion.

2. Materials and methods

2.1. Study area

The study area was Saravan Forestland Park, a forest located in the Guilan province (15 km south of Rasht city, Northern Iran, 37°07'05" N; 49°38'49" E, Figure 1a) at a mean altitude close to 100 m a.s.l., slope between 10 and 40% and southern and northern aspect. The climate of the study area is Mediterranean, Csa type (Hot-summer Mediterranean climate) according to Koppen classification (Kottek et al., 2006). The mean annual temperature and rainfall are 16.3 °C and 1360 mm, respectively. January is the coldest month and August the warmest. Soil texture is prevalently silty clay loam. This park is one of the oldest forestlands in Guilan province with an ample biodiversity (Parhizkar, 2024). Dominant species include *Carpinus betulus*, *Trifolium diffusum*, *Equisetum telmateia*, *Quercus castaneifolia*, *Pinus taeda*, *Parrotia persica*, *Tommasinia verticillaris* and *Populus alba*. On the hillslopes of this park, several plant species have

been lost in recent decades mainly due to deforestation (for installation of high-voltage towers) and wildfires (Parhizkar and Cerdà, 2023). The reduction of forestland cover on these hillslopes (Figure 1c) has led to soil degradation with rill formation (Figure 1d) (Parhizkar et al., 2020c). These rill erosion-prone areas are important to investigate on their morphology and formation mechanism for soil conservation research in the Northern Iran. Therefore, this area seems to be suitable for the aims of soil conservation mentioned in this study.

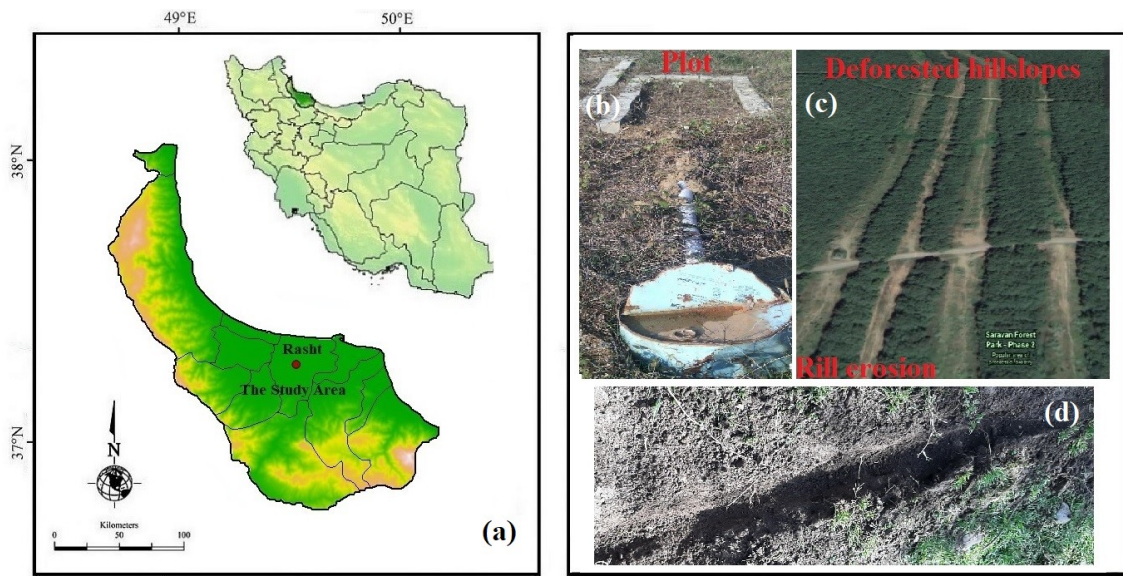


Figure 1 - Geographical location of the study area (a), and pictures of a plot (b), deforested hillslopes (c) and a formed rill (d) (source: Google[®] Map[®]).

2.2. Experimental design and plot construction

The experimental design compared the hydrological and erosive response, and properties of soils inoculated with the *B. arenosi* (hereafter indicated as “treated” soil) and untreated sites (assumed as control). Following previous investigations in the same area (Parhizkar et al., 2020b; 2021a; 2021b), two experimental plots, each one of 3 m in length $\times$ 1 m in width, were installed on a hillslope with a uniform profile (longitudinal slope of 11%) and very similar conditions of soil. The number of experimental plots was low for budget limitations. Each plot was isolated from the remaining area using

concrete blocks, purposely waterproofed and partially inserted into the soil. At the lower side of the plot, a funnel with a height of 20 cm conveyed rainwater through a pipe to a iron tank of 220 litres, in order to collect runoff and sediments. Litter, weeds, and rocks were removed from soil before the experiment start.

The same soil conditions in the two experimental plots allow recognising the effects of inoculation with *B. arenosi* on the hydrological and erosive response, and key properties of soil.

2.3. Soil inoculation with *B. arenosi*

B. arenosi isolate was obtained by Nutrient Agar general medium (Eevers et al., 2015) following the standard protocols reported by Benson (1967). Accordingly, in order to extract the *B. arenosi*, 1 g of soil sample in four repetitions were mixed with 0.85% NaCl solution. Then, 1 ml of this solution was transferred into a glass bottle, and placed in a Petri-dish diameter of 9 cm) in three replications. Finally, number of bacteria was obtained by manually colony counting (Awad et al., 2012).

A pure culture from *B. arenosi* strain stored at -80°C was grown in a broth media of liquid nutrient at 28°C to achieve the suitable density (10^8 Colony Forming Units, CFU, per mL after dilution). The surface of soil in one of the two plots was inoculated with *B. arenosi* (10^9 CFU in 1300 ml), using the spraying technique reported by Wang et al. (2009), mixed by a whisk by hand and then incubated for no less than three months.

2.4. Measurement of soil properties

The soil properties described below were directly measured in fifteen replications, collecting each sample in one point randomly chosen close to the each plot immediately after each recorded rainfall.

2.4.1. Physical properties

The water infiltration rate (IR) was measured using a double-ring infiltrometer (Li et al., 2019). The tensile strength of soil was determined on disks (16 mm in diameter and 9 mm in depth) extracted using a scalpel from the larger soil aggregates in the glass petri dish (Dexter and Kroesbergen, 1985). A mechanical jack was driven upwards at a speed of 0.005 mm/s towards a parallel plate. The load applied by the jack was determined by a digital data-logger device at 2 s intervals. Aggregate failure was observed after the formation of a vertical crack in the disk (Hagen et al., 1995). The mean weight diameter (MWD) was measured by the wet-sieving method (Figure 2).

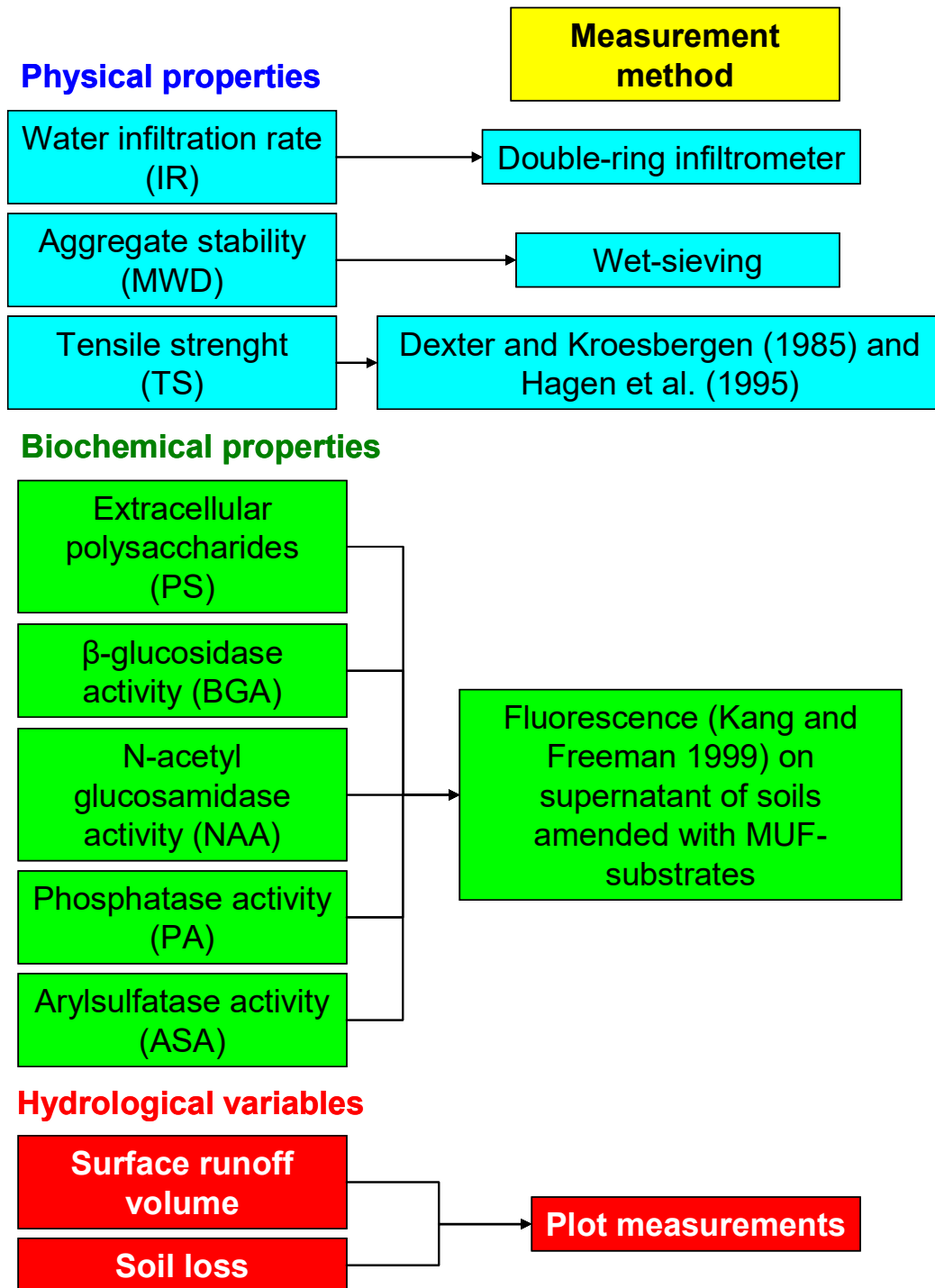


Figure 2 – Variables and measurement methods used in this study.

2.4.2. Biochemical properties

Soluble organic material was removed from soil samples using 0.01 M CaCl₂ (at a 1:10 soil: solution ratio) in polypropylene centrifuge tubes of 50 mL. Then, these tubes were

packed in ice and placed on a shaker for 30 min. After removing the supernatant, the extracellular polysaccharides (PS) was extracted, and its concentration was determined according to the method of Redmile-Gordon et al. (2020). A re-suspending pellet in extraction buffer (2 mM $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, 0.760 g L⁻¹, 4 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.552 g L⁻¹, 9 mM NaCl, 0.526 g L⁻¹, and 1 mM KCl, 0.0746 g L⁻¹), adjusted to pH 7 with 1 M HCl and pre-cooled to 4 °C, was used. Tubes were shaken for 120 min, and centrifuged for a half hour. The supernatant containing extracellular polysaccharide was transferred into other tubes, frozen in liquid N₂, and stored at -80°C prior to analyse the total polysaccharide concentration (Dubois et al., 1956).

Moreover, the activity of four soil enzymes – β -glucosidase (BGA), N-acetyl glucosamidase (NAA), phosphatase (PA), and arylsulfatase (ASA) - was measured on the fresh sieved samples refrigerated at 4 °C. MethylUmbelliFeryl (MUF)-substrates were used as a model for the measurements. MUF-b-D-glucopyranoside (MUF-G), MUF-N-acetyl-b-D-glucosaminide (MUF-N), MUF-phosphate disodium salt (MUF-P) and MUF-sulfate potassium salt (MUF-S) were used for BGA, NAA, PA and ASA, respectively. Soil samples of 1.7 g were amended with 5 mL of substrate. After 1 h of incubation at 20°C, fluorescence in the supernatant was measured, using a fluorometer with a 450 nm emission and 330 nm excitation wavelengths (Kang and Freeman, 1999) (Figure 2).

2.5. Measurement of hydrological variables

Rainfall, runoff and soil loss were monitored at the two plots throughout September 2019 to November 2021 (Figures 1b and 2). The depth and duration of all rainfalls over 5 mm in this period were recorded by an automatic rain gauge installed close to the plots each five minutes. The mean intensity was calculated as the ratio of rainfall depth by its duration. The rainfall depth was between 5.2 and 16.5 mm and the mean intensity between 2.1 and 8.1 mm h⁻¹ (Table 1).

During each rainfall event, the surface runoff volume was manually measured in the tank each five minutes (as for rainfall). The runoff measurement started once the soil was saturated, to avoid that the measurement was biased by unsteady infiltration. Each rainfall-runoff event was divided in three periods to have at least three replications for

surface runoff and soil loss, caring that rainfall in each period was characterized by the same depth and intensity. After oven-drying (at 80 °C for 1 day), the sediment weight in water sample of two litres was measured for each runoff record. The runoff coefficient was calculated as the ratio of the runoff volume by the corresponding precipitation.

2.6. Statistical analysis

First, a one-way ANOVA was applied to the hydrological variables (runoff coefficient and soil loss) and soil properties, considered as dependent or response variables, assuming the soil condition (with two levels, treated and untreated soils) as independent variable or factor. The equality of variance and normal distribution are assumptions of the statistical tests; these assumptions were evaluated by normality tests or were square root-transformed, when necessary.

Second, pairwise comparisons by Tukey's test (at $p < 0.05$) were carried out to identify statistical differences in the response between treated and untreated soils .

Third, the correlation matrix using Pearson's method was computed to explored the reciprocal relationships between pairs of studied variables.

Fourth, a Principal Component Analysis (PCA) was applied, in order to derive a lower number of uncorrelated variables (Principal Components, PCs) (Lee Rodgers and Nicewander 1988) from the original variables, losing as little information as possible. The original variables (expressed by different measuring units) were standardised prior to PCA. A number of PCs explaining at least a percentage of 70% of the original variance was retained (Lee Rodgers and Nicewander 1988).

Fifth and finally, the observations related to the hydrological variables and soil properties were grouped in clusters using Agglomerative Hierarchical Cluster Analysis (AHCA), a distribution-free ordination technique to group samples with similar

characteristics by considering an original group of variables. As similarity-dissimilarity measure the Euclidean distance was used.

The statistical analysis was carried out using the XLSTAT software (release 2019, Addinsoft, Paris, France).

3. Results

ANOVA shows that all studied variables were significantly different between treated and untreated soils (Table 2).

Table 1 – Results of one-way ANOVA applied to hydrological variables as well as to properties of soils measured at the experimental plots throughout the monitoring period (Saravan Forestland Park, Guilan, Northern Iran).

Factor (levels)	Sum of squares	Mean squares	F	Pr > F
Soil condition (treated/untreated soil)	IR			
	92.541	92.541	65.608	< 0.0001
	TS			
	6.816	6.816	436.412	< 0.0001
	MWD			
	0.335	0.335	180.180	< 0.0001
	PS			
	6192.033	6192.033	139.565	< 0.0001
	BGA			
	154.224	154.224	171.529	< 0.0001
	NAA			
	771.248	771.248	387.322	< 0.0001
	PA			

	119.481	119.481	145.045	< 0.0001
	ASA			
	0.625	0.625	83.102	< 0.0001
	RC			
	0.459	0.459	33.565	< 0.0001
	SL			
	320.329	320.329	14.106	0.001

Notes: IR = water infiltration rate; MWD = mean weighted diameter; TS = tensile strength; PS = polysaccharides; BGA = β -glucosidase activity; NAA = N-acetyl glucosamidase activity; PA = phosphatase activity; ASA = arylsulfatase activity; RC = runoff coefficient; SL = soil loss.

3.2. Changes in physical and biochemical properties of soil due to inoculation

IR, equal to $4.17 \pm 0.29 \text{ mm h}^{-1}$ in the untreated soil, increased to $7.68 \pm 0.32 \text{ mm h}^{-1}$ after the treatment. TS and MWD of the treated soils were higher ($1.55 \pm 0.03 \text{ kPa}$ and $0.54 \pm 0.01 \text{ mm}$, respectively) compared to the untreated plot ($0.60 \pm 0.03 \text{ kPa}$ and $0.33 \pm 0.01 \text{ mm}$) (Figure 3).

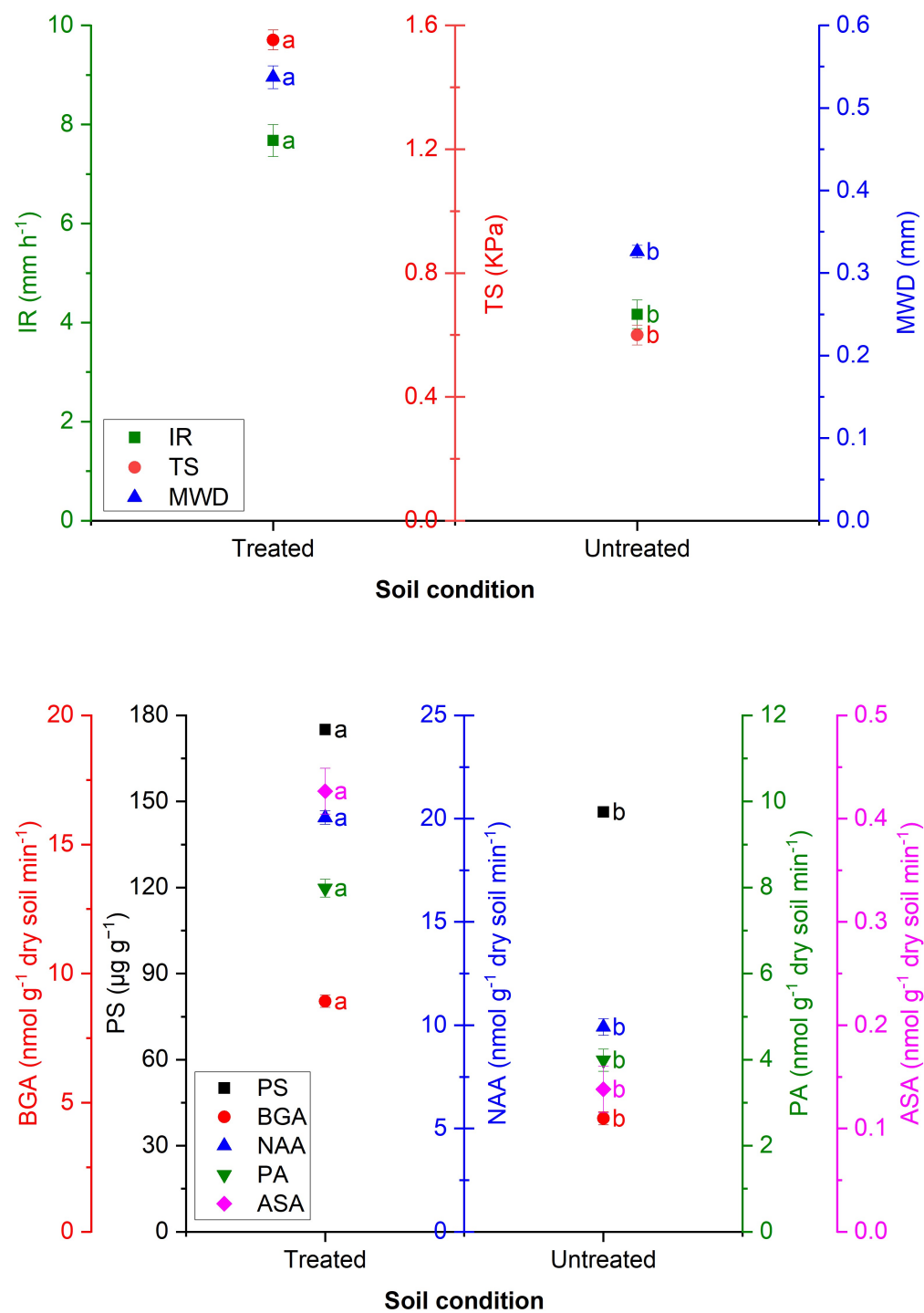


Figure 3 – Mean $\pm$ standard error ($n = 15$) of the properties of soils measured at the experimental plots throughout the monitoring period (Saravan Forestland Park, Guilan,

Northern Iran). *Legend: IR = water infiltration rate; MWD = mean weighted diameter; TS = tensile strength; PS = polysaccharide; BGA = β -glucosidase; NAA = N-acetyl glucosamidase; PA = phosphatase; ASA = arylsulfatase. Different letters indicate significant differences after Tukey's tests ($p < 0.05$).*

The treated soils showed higher values of extracellular polysaccharide and all enzymes compared to the untreated plot. In more detail, PS of the untreated soil ($146 \pm 1.67 \mu\text{g g}^{-1}$) increased to $175 \pm 1.677 \mu\text{g g}^{-1}$ in the treated plot, while, in the latter soil condition, the enzyme contents of the treated soil was over 2-fold the value measured in the control (Figure 3).

3.2. Changes in hydrological response of soil due to inoculation

RC was on average 0.55 ± 0.15 and 0.30 ± 0.07 for the untreated and treated soils, respectively. Mean SL was higher in the untreated plot ($148 \pm 1.68 \text{ g m}^{-2}$) compared to the treated soil ($8.30 \pm 2.91 \text{ g m}^{-2}$) (Table 2).

- 1 Table 2 – Values of the hydrological variables measured at the experimental plots throughout the monitoring period (Saravan Forestland
2 Park, Guilan, Northern Iran).
3

Date	Rainfall			Runoff coefficient		Soil loss (g m ⁻²)	
	depth (mm)	duration (h)	mean intensity (mm h ⁻¹)	Untreated	Treated	Untreated	Treated
07 October 2019	13.5	1.82	7.42	0.58	0.33	22.21	12.01
20 October 2019	6.1	1.69	3.61	0.59	0.37	9.54	5.52
13 November 2019	12.7	1.99	6.38	0.63	0.32	17.14	7.92
09 December 2019	10.1	1.47	6.87	0.71	0.42	17.25	8.15
14 January 2020	12.3	2.83	4.35	0.42	0.27	11.85	7.89
08 February 2020	8.6	2.02	4.26	0.44	0.24	10.81	6.28
18 October 2020	8.3	1.03	8.06	0.95	0.45	28.94	15.24
12 November 2020	11.9	2.10	5.67	0.46	0.27	13.47	8.47
24 December 2020	16.5	2.11	7.82	0.50	0.24	22.54	12.15
23 February 2021	11.7	1.89	6.19	0.48	0.28	17.05	8.84
10 October 2021	11.1	1.98	5.61	0.49	0.24	14.09	9.23
21 October 2021	6.9	2.25	3.07	0.47	0.30	8.65	5.17
04 November 2021	5.2	2.47	2.11	0.61	0.33	7.21	5.04
15 November 2021	9.5	1.82	5.22	0.57	0.27	12.49	6.39

4

30 November 2021	14.1	3.19	4.42	0.31	0.17	9.27	6.18
Mean	10.57	2.04	5.40	0.55	0.30	14.83	8.30
Standard deviation	3.16	0.52	1.77	0.15	0.07	6.08	2.91

5

6 3.3. *Correlation and multivariate analysis*

7

8 Pearson's matrix shows a high and positive correlation between RC and SL ($r = 0.74$).

9 Moreover, RC was inversely correlated to IR ($r = -0.60$), which in turn was positively

10 associated to MWD ($r = 0.78$). Also the coefficients of correlation between PS and all

11 soil enzymes on one side, and MWD on the other side were high and positive ($r > 0.83$)

12 (Table 3).

13 Table 3 - Pearson's correlation matrix of hydrological variables, and soil properties and enzymes measured at the experimental plots
 14 throughout the monitoring period (Saravan Forestland Park, Guilan, Northern Iran).

15

Variables	RC	SL	IR	TS	MWD	PS	BGA	NAA	PA	ASA
RC	1	0,74	-0,60	-0,73	-0,72	-0,76	-0,75	-0,74	-0,71	-0,72
SL		1,00	-0,39	-0,60	-0,56	-0,66	-0,59	-0,53	-0,63	-0,60
IR			1,00	0,83	0,78	0,69	0,75	0,87	0,69	0,60
TS				1,00	0,90	0,90	0,89	0,94	0,91	0,80
MWD					1,00	0,88	0,88	0,88	0,87	0,83
PS						1,00	0,86	0,90	0,86	0,83
BGA							1,00	0,91	0,87	0,85
NAA								1,00	0,86	0,82
PA									1,00	0,90
ASA										1,00

16

17 Notes: RC = runoff coefficient; SL = soil loss; IR = water infiltration rate; MWD = mean weighted diameter; TS = tensile strength; PS = polysaccharide; BGA = β -
 18 glucosidase activity; NAA = N-acetyl glucosamidase activity; PA = phosphatase activity; ASA = arylsulfatase activity. Red and green colours refer to negative and
 19 positive values, respectively.

Among the PCs calculated by the PCA, the first component (PC) alone explains approximately 80% of the total variance of the original variables, while the other PCs explain much lower values of this variance. All soil enzymes are associated to the PC1 with very high loadings (> 0.812), while the loadings of RC and SL on this PC are negative and noticeable (absolute value > 0.694) (Figure 4). The scores of the observations on PC1 are significantly different between the two soil conditions, and are clustered in two distinct groups by AHC (Figure 5).

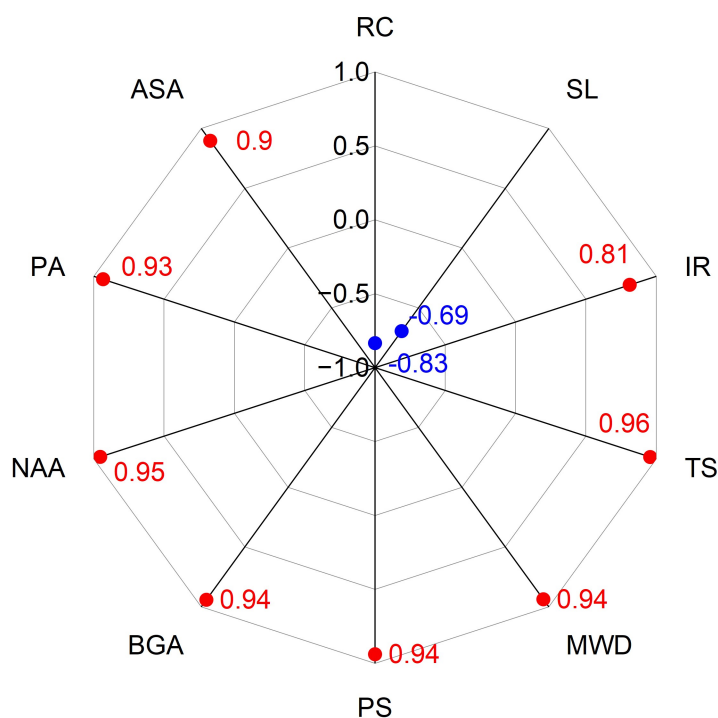
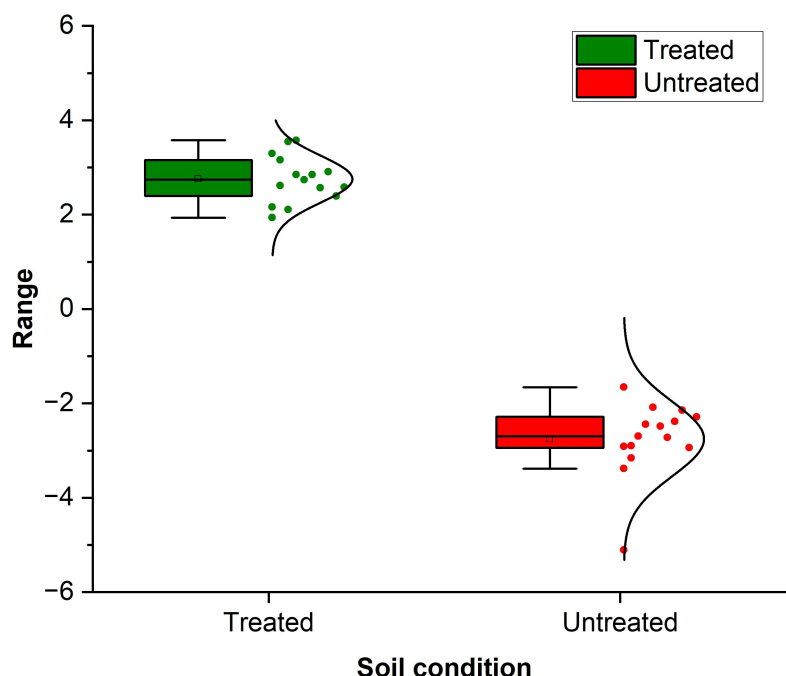


Figure 4 – Loadings of hydrological variables and soil properties on the first Principal Component calculated by the Principal Component Analysis based on experiments at plots (Saravan Forestland Park, Guilan, Northern Iran). *Legend: IR = water infiltration rate; MWD = mean weighted diameter; TS = tensile strength; PS = polysaccharides; BGA = β -glucosidase activity; NAA = N-acetyl glucosamidase activity; PA = phosphatase activity; ASA = arylsulfatase activity; RC = runoff coefficient; SL = soil loss. Red and green colours refer to negative and positive values of PC1, respectively.*



38

39 Figure 5 – Boxplot (showing the main statistics) and distribution of the first Principal
 40 Component calculated for the two soil conditions (treated and untreated soils) by the
 41 Principal Component Analysis based on experiments at plots (Saravan Forestland Park,
 42 Guilan, Northern Iran).

43

44 4. Discussions

45

46 4.1. Changes in physical and biochemical properties of soil due to inoculation

47

48 Soil inoculation with *B. arenosi* significantly increased the tensile strength (+159%) and
 49 stability of soil aggregates (MWD = +64.8%). Many other investigations have reported
 50 that soil microorganisms noticeably increase both these properties (e.g., Ingham, 2009;
 51 Jensen et al., 2019; Tisdall et al., 2012). According to the literature, bacteria adhere to
 52 fine particles of soil, thanks to their sticky and resistant cell walls and PS sheaths

(Bruinsma et al., 2001; Tripathi et al., 2012). This effect lets bacteria absorb water (Huang et al., 2002) and bind soil particles (binding effect) (Borges et al., 2008). PS together with glycoproteins produced by bacteria can coat the surface of soil particles and strengthen its structure by this binding effect (Cania et al., 2019; Erktan et al., 2020; Ingham, 2009). Moreover, soil aggregation due to PS addition with bacteria shifts micro-aggregates to macro-structures (bounding effect) (Colica et al., 2014), which leads to the formation of vertical micro-channels in the soil surface layer and thereby increases water infiltration (Sadeghi et al., 2017). According to Carrasco et al. (2009), soil aggregate stability can increase by 45% after inoculation of bacteria in soils, and this increase is in line with other studies that measured soil aggregate stability using MWD (Ren et al., 2022; Ghasemzadeh et al., 2023). The latter authors even demonstrated a high correlation ($R^2 = 0.74$) between MWD and extracellular PS concentration.

The increase in soil aggregate stability in the inoculated soils thanks to the extracellular PS concentration indicates that the latter is one of the most significant binding factor in soil (Tisdall and Oades, 1982). Therefore, PS can be considered as an indicator of formation of soil aggregates with increases in tensile strength (Xie et al., 2007). The binding effect due to the bacteria action on soil particles and aggregates, which become strongly connected after soil inoculation, also increases TS, and this makes the soil more resistant against raindrops and overland flow (Carrasco et al., 2009; Kheirfam et al., 2020; Sadeghi et al., 2023). In this study, the reciprocal associations among the physical properties of soil are confirmed by the high correlations between MWD, TS and PS.

Moreover, the relationships among the physical properties, PS and enzyme activities are also worth to be mentioned, and this fills the research gaps of current studies about field measurements of alterations in enzyme activities, PS concentration and other properties in soil subject to erosion in response to treatment with *B. arenosi*. The differences in enzyme activities between the inoculated and untreated soils can be ascribed to the

amount of organic matter and its decomposition rate, which both increase in treated soils due to the presence of bacteria, as reported by Park et al. (2014). The PS also contain non-negligible amounts (up to 25%) of organic matter, which improves the soil properties and resulting quality and structure (Chamizo et al., 2019; Sadeghi et al., 2020). Soil micro-organisms also play an essential role in sequestering carbon (Nisha et al., 2007) with a carbon storage capacity that can be amplified by 1000 times (Kaplan et al., 1994) compared to untreated soils (Sears and Prithiviraj, 2012; Keirfan et al., 2020). Soil inoculation with *B. arenosi* also results in beneficial effects on water infiltration rate, which increased by 84.3% and thus significantly compared to the untreated soils. This value is close to the increase in infiltration (74%) measured by Jafarpoor et al. (2022) after soil inoculation with cyanobacteria. Also, Sadeghi et al. (2023) reported that soil inoculation with bacteria results in 1.5-fold water infiltration volume compared to the untreated control. These increases must be ascribed not only to the aforementioned improvements in soil aggregate stability, but also to the formation of biofilm layers, both on the surface and around the particles of the treated soil, through PS secretion (Huang et al., 2002; Valencia, 2014). These biofilms are able to absorb and retain water into soil, and this may appear as an increase in water infiltration.

4.2. Changes in hydrological response of soil due to inoculation

The low water infiltration capacity of soils is an important issue in Mediterranean ecosystems, where the intense rainfall events may increase the overland flow and soil loss (Lucas-Borja et al., 2018). Considering this key role of infiltration on the hydrological response of semi-arid soils, the application of microorganisms, in particular direct inoculation of bacteria, may be theoretically advisable to increase water infiltration in soil, and, as a consequence, to reduce runoff generation and soil erosion. This is confirmed in this study, since the soil treatment with *B. arenosi* clearly resulted in significant decreases (on average 45%) in surface runoff and soil loss in comparison to the untreated sites. This decrease in runoff coefficients is in line with other studies,

which report reductions by 12% (Zhao and Xu, 2013) to 75% (Belnap et al., 2013) in runoff volume in soils with a well-developed biological soil crust, due to their inoculation with bacteria. According to Sadeghi et al. (2017), bacteria reduce the overland flow by directly absorbing water as well as increasing infiltration, and this reduction increases with microbial biomass in soil. The decrease in surface runoff due to enhanced infiltration is mainly due to Horton's mechanism governing runoff generation, which is typical of semi-arid soils (Horton, 1933; Sadeghi et al., 2023).

The literature is again in close agreement with the noticeable reduction in soil loss measured in this study after soil inoculation with *B. arenosi*. For instance, compared to an untreated soil, this treatment decreased soil loss by 80-90% and sediment concentration by 60-75% after soil inoculation with bacteria in the study carried out by Sadeghi et al. (2020). Inoculation of cyanobacteria reduced soil loss and sediment concentration by 82 and 62%, respectively, in the investigation by Jafarpour et al. (2022). Kheirfam et al. (2020) found decreases in soil erosion between 36 and 57% in soils treated with cyanobacteria, and this reduction was even 100 to 270-fold the background erosion measured in untreated soils (Sadeghi et al., 2023). Undoubtedly, the reduced erosion due to soil inoculation with *B. arenosi* derives from the increase in water infiltration and reduction in surface runoff, which lead to a consequent decrease in soil detachment due to the overland flow. Another important effect of the inoculated bacteria contributing to decrease the soil loss is the presence of bacteria cell walls that act as a micro-mulch layer, thus decreasing rainsplash erosion (Kheirfam et al., 2017; Williams et al., 2018; Kheirfam et al., 2020).

4.3. Correlation and multivariate analysis

The direct contribution of infiltration rates, tensile strength and aggregate stability of soil to decrease the hydrological response in inoculated soils is also demonstrated by the noticeable correlations between the hydrological and physical parameters of soil found

in this study. These results are consistent with several past investigations, which attributed the decreases in runoff coefficient and soil loss to the increases in water infiltration, and, more in general, to changes in other physical properties of soil. For instance, Abid et al. (2009) and Bombino et al. (2019) demonstrated that the increases in infiltration capacity, aggregate stability and tensile strength of soil play a beneficial effect on runoff generation, and, indirectly, on soil erosion. However, if the decrease in runoff generation due to the increase in water infiltration rate is expected (e.g., Wei et al., 2019), the reduction in erosion rates and erodibility of soil is an indirect effect of the increased infiltration due to the soil treatment (Wang et al., 2015). Therefore, soil erosion can be considered as a function of both runoff volume and infiltration (Loáiciga and Johnson, 2018), and this is further confirmed by Arabi et al. (2008), who reported a negative correlation between water infiltration and soil loss.

The multivariate statistical analysis identified a meaning derivative variable (the first Principal Component), which can be considered as a synthetic measure of key hydrological, physical and biochemical properties of soil governing its erosive response. Moreover, the PC1 clearly and sharply discriminates inoculated from untreated soil. This agrees with findings of Parhizkar et al. (2023a), who found that a first principal component of PCA reveals obvious discrimination in soil erodibility between inoculated and untreated soils.

4.4. Practical implications

The main implication of this study is a proof that the effects of soil inoculation with *B. arenosi* are able to noticeably change the hydrological response of soil through strong but beneficial modifications of key properties. In other words, extracellular polymeric substances secreted by *B. arenosi* and increases enzymatic activities of soil improve soil structure with formation of stable aggregates (Zhang et al., 2021a) with a consequent increase of water infiltration, decrease in runoff generation and soil erodibility (Costa et al., 2018; Totsche et al., 2018). This is an important result, which highlights that the associations between soil physics (with a particular focus on soil hydrology) and microbial community structure are of great interest for land managers charged with the delicate task to increase soil resistance to detachment, erosion being one of the most

important concerns of forest ecosystems. Therefore, the use of selected microbial strain may be adopted as an effective action to reduce soil erodibility in delicate environments as forestlands (Charman and Murphy, 2007). More in general, a broader adoption in soil conservation practices based on inoculation with microorganisms (especially bacteria) may limit soil degradation in agro-forest areas and support sustainable systems for agricultural and wood production (Nannipieri et al., 2003). This impact significantly supports the improvement in soil properties and contrasts other harmful conditions for soil health and quality.

5. Conclusions

This study has evaluated whether and to what extent soil inoculation with *B. arenosi* is able to significantly change key physical and biochemical properties of soil, and runoff and erosion rates through measurements at small plots under natural rainfalls in degraded forestlands of Northern Iran.

Water infiltration, tensile strength and aggregate stability of soil were significantly different between treated and untreated soils. Moreover, polysaccharides and enzymes of soil were significantly higher after soil inoculation compared to the untreated soils. A noticeable decrease in both surface runoff and soil loss in the inoculated soils resulted from those changes. The reciprocal associations between the hydrological response of soil on one side, and the measured physical and biochemical variables on the other side were clearly revealed by the correlation analysis.

The multivariate analysis using Principal Component Analysis and Agglomerative Hierarchical Cluster Analysis provided a meaningful parameter, which synthesises all studied variables and discriminate the inoculated soils from the untreated sites.

Overall, the results of this investigation suggest that soil inoculation with selected bacteria (such as *B. arenosi* as in this case study) may have a significant potential for reducing soil erosion under the experimental conditions. Therefore, this practice may be considered as a bio-based technique for ecosystem restoration in degraded forestlands and, more in general, in sites affected by severe soil degradation. More research is, however, needed to test the capacity of other soil microorganisms to improve the soil's hydrological response, preferably working on larger spatial scale (hillslopes rather than

small plots, as in this study) and higher rainfall intensities (simulating the effects of the ongoing climate change scenarios).

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