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## Geomorphic connectivity as a driver for the soil bioindicator distribution, implications for future research and modeling

**Abstract:** Pelacani S., Raspini F., Roccotelli A., Barbadori F., Ceccherini M.T., Tommasini S., Moretti S., *Geomorphic connectivity as a driver for the soil bioindicator distribution, implications for future research and modeling*. (IT ISSN 0391-9838, 2025). Hydrological structural connectivity, the degree to which water can move across a landscape, significantly influences water and sediment transport. While its impact on these processes is well-established, the extent of its influence on soil biological activity is still being researched. This study examines the link between geomorphic connectivity (CI) and soil microbial functional indicators in the Greve watershed, Tuscany (Italy), by developing a new weighted connectivity index ( $CI_{FB}$ ) that integrates the fungi-to-bacteria (F:B) abundance ratio as a bioindicator. Using high-resolution geospatial data, rare earth elements (REEs), satellite indices (NDVI, NDWI), and machine learning (Stochastic Gradient Boosting, SGB), were modeled relationships between geomorphic variables, soil erodibility (K factor), and microbial indicators. Results showed that CI ranged from -9.0 and +0.6, but the weighted index,  $CI_{FB}$ , ranged from -10.0 and +4.0. This showed that the F:B ratio weighting caused an increase in the range of connectivity values between landform units, which implies it strengthened the relationships between them. The key finding is that  $CI_{FB}$  can better identify hidden areas of sediment coupling and decoupling within a landscape. Under the physiographic environmental variables of the study area, the spatial distribution of F:B ratio was mainly related to CI, flow path length and K factor. The proposed approach, while requiring further development, show great potential for monitoring and conserving ecosystems, especially within watersheds, not just in the Mediterranean region but also in other vulnerable areas.

**Key words:** Biogeomorphology, Ecogeomorphic system, Hydrological structural connectivity, Sediment Transport Capacity, Soil Erodibility, Fungi to Bacteria ratio.

**Riassunto:** Pelacani S., Raspini F., Roccotelli A., Barbadori F., Ceccherini M.T., Tommasini S., Moretti S., *La connettività geomorfica come fattore determinante per la distribuzione dei bioindicatori del suolo, implicazioni per la ricerca e la futura modellazione*. (IT ISSN 0391-9838, 2025). La connettività strutturale idrologica, ovvero il grado di mobilità dell'acqua in un territorio, influenza significativamente il trasporto di acqua e sedimenti. Sebbene il suo impatto su questi processi sia ben noto, la portata della sua influenza sull'attività biologica del suolo è ancora oggetto di ricerca. Questo studio esamina il legame esistente tra la connettività geomorfologica (CI) e le comunità microbiche del suolo per il bacino idrografico della Greve, in Toscana (Italia), sviluppando un nuovo indice di connettività ponderato (CIFB) che integra il rapporto dell'abbondanza funghi/batteri (F:B) come bioindicatore. Utilizzando dati geospatiali ad alta risoluzione, elementi delle terre rare (REE), indici satellitari (NDVI, NDWI) e apprendimento automatico (Stochastic Gradient Boosting, SGB), sono state modellate le relazioni esistenti tra le variabili geomorfologiche, l'erodibilità del suolo (fattore K) e gli indicatori microbici. I risultati hanno mostrato che l'indice di connettività (CI) varia da -9,0 a +0,6, mentre l'indice ponderato, CIFB, varia da -10,0 a +4,0. Ciò dimostra che utilizzando il rapporto F:B come indice di ponderazione per CI, si assiste ad un aumento dell'intervallo dei valori di connettività, il che rafforzerebbe le relazioni tra le unità di paesaggio. La scoperta chiave è che il CIFB è in grado di distinguere meglio tra aree in cui i sedimenti si depositano e aree in cui vengono trasportati. In base alle caratteristiche fisiografiche dell'area di studio, la distribuzione spaziale del rapporto F:B è principalmente correlato a CI, alla lunghezza del percorso del deflusso e al fattore K. L'approccio proposto, pur necessitando di ulteriori sviluppi, risulta essere promettente ai fini del monitoraggio e della conservazione degli ecosistemi a scala di bacino idrografico, sia in ambiente Mediterraneo che in altre aree vulnerabili.

**Termini chiave:** Biogeomorfologia, Eco-geosistema, Connnettività strutturale idrologica, Capacità di trasporto di sedimenti, erodibilità del suolo, rapporto Funghi-Batteri.

### INTRODUCTION

Climate change is causing significant intensifications in hydrological events, such as floods, droughts and typhoons, altering their frequency, intensity and spatial distribution. These extreme hydrological events pose significant challenges to water resources management, natural infrastructure resilience, and the risk of biodiversity loss (UNESCO, 2020).

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Future climate scenarios for the Mediterranean region predict more frequent droughts; in fact, it has been estimated that the 100-year drought will occur 10 times more frequently in the near future (Weiß *et al.*, 2007).

It is therefore necessary to implement mitigation measures that promote sustainable land use, capable of limiting the impacts of global warming on water resources, ecosystems and communities (Chiriacò *et al.*, 2025). To find and suggest effective solutions that promote environmental sustainability, it is essential to study the interaction between abiotic and microbial bioindicators, because these interactions influences geomorphological and ecological processes (Naylor *et al.*, 2002; Holt and Miller, 2010). Bioindicator microorganisms are able to provide information on past climatic events and current soil dynamic processes (Ludwig *et al.*, 2018). Few studies have investigated sediment thousands of years old, focusing on sedimentary microbial DNA for climate-environment-ecosystem reconstruction (Jia *et al.*, 2022; Li *et al.*, 2024; Wang *et al.*, 2025). Ancient microbial communities can give evidence of paleoecological events such as floods. From the observation made in the Constance Lake in the Alpine region, this microbial evidence indication corroborates the flood history found into their sedimentary layers, making microbial analysis a valuable tool for paleoclimatology (Wang *et al.*, 2025). Metagenomic analysis of sedimentary ancient DNA (sedaDNA) from a site in Norway has provided detailed insights into human land use from the late Bronze Age to the Medieval period, revealing changes in microbial and plant diversity and human impact over time. This research shows that sedaDNA offers a high-resolution complement to traditional methods like pollen and macrofossil analysis, and its successful application demonstrates potential for reconstructing dynamic landscapes and understanding long-term human-environment interactions in Scandinavia (La Torre *et al.*, 2025). The diversity of life on Earth is largely due to fungi and bacteria (de Boer *et al.*, 2005). They collectively accounting for about 90% of biomass in soil (Beule *et al.*, 2019). These organisms play crucial roles in nutrient cycling, mineralization, and soil health. Bacteria are particularly involved in the transformation of soil organic carbon (SOC), while fungi are known for decomposing mineral components like soil phosphorus (Zhu *et al.*, 2022). Microorganisms are sensitive to environmental stressors such as erosion, and their assemblages and activities can reflect the past and present soil health, making them valuable bioindicators (Ma *et al.*, 2022). The “memory” of past events in soil microbial assemblages is driven by a combination of factors, including the persistent presence of soil organic matter (SOM) composition, genetic adaptation within microbial communities, and the formation of stable microbial associations linked to specific soil properties (Bernal *et al.*, 2023; Wilhelm *et al.*, 2023). According to the National Resources Conservation Service (NRCS, 2015;

[https://www.nrcs.usda.gov/sites/default/files/2022-10/biological\\_indicators\\_overview.pdf](https://www.nrcs.usda.gov/sites/default/files/2022-10/biological_indicators_overview.pdf)), soil erosion legacy can be captured by bioindicator microorganisms. Studies showed that badlands have reduced microbial diversity and microbial network complexity compared to lesser eroded soils, indicating an increase in the relative abundances of some bacterial families involved in N cycling, such as *Aacetobacteraceae* (Guida *et al.*, 2022; Qui *et al.*, 2021).

Soil erosion by water is a major geomorphological hazard and driver of land degradation worldwide, and its management has become an increasingly challenging issue especially in Mediterranean area. Thus, understanding and quantifying soil erosion and channel-type processes is essential to managing soil and water conservation, and can be achieved by using the geomorphic connectivity approach (Wainwright *et al.*, 2011; Turnbull *et al.*, 2018). Three types of geomorphic connectivity have been reported in the literature: (i) landscape connectivity, (ii) hydrological connectivity, and (iii) sediment connectivity (Wohl *et al.*, 2019). All of them have a structural and a functional intrinsic components (Wainwright *et al.*, 2011; Turnbull *et al.*, 2018). The sediment connectivity approach supports both scientific studies and applied questions, by allowing researchers to quantify the pathways and patterns of sediment transfer in a landscape (EC, 2022. *Integrated sediment management Guidelines and good practices in the context of the Water Framework Directive*. Available at: [https://environment.ec.europa.eu/system/files/2022-09/CISdocuments/sedimentfinalTO\\_BE\\_PUBLISHED\\_1430554724.pdf](https://environment.ec.europa.eu/system/files/2022-09/CISdocuments/sedimentfinalTO_BE_PUBLISHED_1430554724.pdf)). To quantify the spatial-temporal changes of the sediment-flow relationship in different geomorphological units (connectivity/dis-connectivity), the Index of Connectivity approach (CI, Borselli *et al.* 2008) has been widely employed. It refers to the functional connectivity between elements of the catchment, for example flow paths or variable source areas. For each pixel, this dimensionless index  $[-\infty, +\infty]$  take into account the combined effect of the up-slope topographic characteristics and land-use types, and the value of these parameters throughout the flow path line a soil particle must travel to reach the nearest stream or sink. Modified versions of the CI related to the calculation of morphometric parameters and the weighted landscape parameter (W) have been applied and demonstrated in different worldwide watersheds, e.g. in Australia (Vigiak *et al.*, 2012; Asadi *et al.*, 2023), in Spain (López-Vicente *et al.*, 2013), in Italy (Pelacani *et al.*, 2008; Cavalli *et al.*, 2013), in Mexico (Ortíz-Rodríguez *et al.*, 2017) and in Japan (Evrard *et al.*, 2013; Chartin *et al.*, 2017). However, none of these studies have used bioindicators to model the CI. We hypothesized that the use of the new W factors based on the fungi to bacteria abundance ratio (F:B) will improve the characterization of the actual soil processes and gain a better understanding of the past and present soil health. The F:B ratio is a recognized indicator of soil health, as the ratio

reflects complex interactions influenced by land management and environmental factors (Djemiel *et al.*, 2023). The ability of soil ecosystems to self-regulate may be reflected in this correlation. Soil erosion can be reduced by the presence of stable soil aggregates, the formation of which can be induced by fungal mycelium (Zhang *et al.*, 2020) and by bacteria through the synthesis of adhesive polysaccharides (Sadeghi *et al.*, 2023). Some studies conducted on an experimental scale have shown that bacterial polysaccharides can reduce the soil loss due to water erosion by up to 98% (Kheirfam *et al.*, 2017), and the development growth stages of rills (Sadeghi *et al.*, 2023; Yang *et al.*, 2023) by influencing the overland flow (Horton, 1933).

Recent microbial studies have introduced a new concept of community coalescence (Mansour *et al.*, 2018) to deepen the combined influence between physical and environmental factors and the microbial community. This approach has been used to study ecological issues such as the hydrological connectivity between the riverbank infiltration and the aquifer (Fillinger *et al.*, 2021), the dissolved organic matter and microbial activity in river-floodplains systems (Meyer *et al.*, 2024).

Physical transport, sediment mixing, and organic matter (OM) redistribution strongly influence microorganism distribution at the watershed scale by moving them, altering their environment, and changing their food sources (Cho *et al.*, 2016). Physical transport moves microorganisms, sediments, and OM, while sediment mixing and OM redistribution control the availability of nutrients and energy sources for microbial communities.

In this study, we aim to investigate the relationship between the hydrological structural connectivity and the fungi to bacteria ratio as bioindicator for a Mediterranean drainage basin in a machine learning framework (ML). To the best of our knowledge, this is the first comprehensive study of such a kind. In this work, we introduce bioindicator microorganisms as a new factor for the analysis of the soil erosion impact, given that they can be considered as a “memory” of soil changes.

The relationships between geomorphic connectivity and bioindicator microorganisms will help us to answer two fundamental questions:

- (i) What is the impact of the hillslope hydrological connectivity index in terms of sediment redistribution?
- (ii) Is there an implication on erodibility factor ( $K$ ) as a key response variable?

The  $K$ -factor was considered in this study since it is recognized as an essential component in the estimation of soil loss by indicating the susceptibility of soil to detachment and transport. Hence, the overall objective of this study is to test whether the CI approach can simulate bio-thermodynamics and physic thermodynamics by modelling the mass and energy redistribution within the landscape. The approach used, by integrating physical processes and bio-

logical system, wants to better understand how mass and energy are redistributed through all geomorphic zones in a landscape. This approach moves beyond studying individual processes in isolation to see how they are interconnected within the entire geomorphic system. This new concept of the eco-geomorphic connectivity indicator, where the F:B ratio was used as a weighting factor, can be used to measure environmental changes as biological response indicators, including those related to changes in temperature, pollution or other environmental stressors by revealing specific biological responses.

## STUDY AREA

### *Geological setting of the study area*

In this study we focused on the Greve stream watershed with a drainage area of 272 km<sup>2</sup>, a left tributary of the Arno river, located in the Chianti area about 10 km south to southwest of Florence, Tuscany, Italy (fig. 1). The altitude ranges between 71 m and 913 m a.s.l. (fig. 1c). The Greve watershed shows a typical Mediterranean climate with long dry summers and wet winters, with a mean annual precipitation of more than 930 mm y<sup>-1</sup> and two rainfall peaks, one in autumn and one in spring. The average annual value of rainfall erosivity (R factor), considering the period 1996-2010, ranges from 922.6 to 2,799.1 MJ·mm·ha<sup>-1</sup>·h<sup>-1</sup>·y<sup>-1</sup>, with a mean value of 1,425.6 MJ·mm·ha<sup>-1</sup>·h<sup>-1</sup>·y<sup>-1</sup> (Napoli *et al.*, 2016). Recently, heavy rainfall events have drastically increased their erosivity with value reaching 480 MJ·mm·ha<sup>-1</sup>·h<sup>-1</sup> in the 15 August 2022. This heavy rainfall caused a flash flood that also affected the first and second order river network.

In the watershed there are three non-metamorphic tectono-sedimentary units outcropping (fig. 1b), belonging to the Ligurian domain (limestone and clayey marly units of Morello Formation and limestone and shales of Sillano Formation, Eocene) (Festa *et al.*, 2010) and Tuscan domain (sandstone unit of Macigno Formation, Eocene-Miocene) (Merla, 1969). A post-orogenic (Plio-Pleistocene) fluvial deposits are especially present on the western flanks of the watershed.

### *Drainage network and Geomorphology*

The actual drainage network of the Greve watershed is characterized, as regards the main course, by a predominantly NW-NE oriented flow that drain into the Arno River (fig. 1c).

The drainage network of the study area has been affected by several tectonic/stratigraphic domains, divided by transversal lineaments and belts that are parallel to the main Northern Apennines transversal tectonic lineaments (Pascucci *et al.*, 2007). A series of WSW-ENE or SSW-NNE

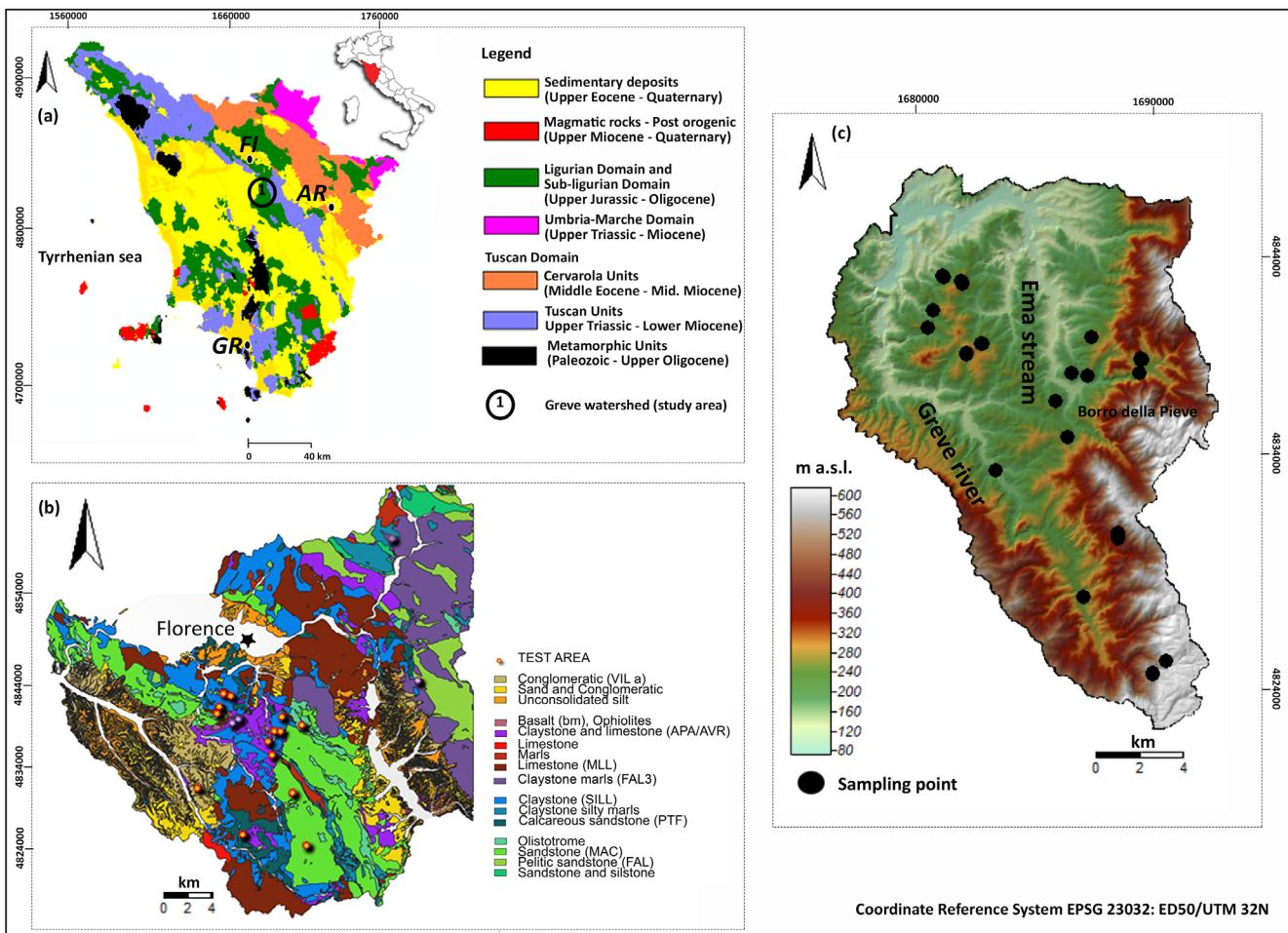


Figure 1 - (a) Lithologic map of Tuscany. (b) Lithologic map of the Chianti Mts. (c) Digital elevation model of the Greve basin (Florence, Italy) and the location of soil site sampling.

tectonic lineaments, representing strike-slip faults, bounds the structural and stratigraphic domains (fig. 1b). It was supposed that these elements are an interposed inheritance between the pre-collisional normal faults that controlled the facies distribution during the “Scaglia Toscana” Fm. deposition and the syn-collisional transpressional faulting that developed the Chianti Anticline (Benvenuti *et al.*, 2014).

Streams are largely parallel to local structures such as faults. The Eastern part of the catchment is characterized by a dendritic network with oval shape sub-basins. The Ema stream is characterized by convex gentle slopes especially where the stream drains towards the Ligurid outcrops. The eastern side and upper part of the Ema basin where the drainage network is developed on Tuscan nappe formations is mainly dominated by fluvial and slope processes. The related morphosculptural landforms are shaped and incised by approximately SW-NE oriented valleys, resulting in narrow crests.

Compared to the eastern sub-catchments, western ones, the rectangular basin shape of the main Greve stream is characterized by trellis networks, with initial river flows

in NW direction and subsequently in NE direction. This marked change in flow direction, or hairpin turn, is accompanied by a large increase in river gradient and local relief. This rectangular basin is characterized by an asymmetric location of the main drainage within the basin.

On the western flank of the Chianti range, rivers generally flow perpendicular to local structures and cross major range-bounding faults. Furthermore, several points along the range crest are characterized by high-elevation, low-relief surfaces. Nonetheless the main drainage divide (MDD) and topographic crests are largely coincident. There are several regions where the MDD and crests are offset (fig. 1c “Borro della Pieve”).

## MATERIAL AND METHODS

### *Modelling of a new index of flow and sediment connectivity*

The sediment connectivity index (CI) proposed by Borselli *et al.* (2008) shows the connection between different parts of the catchment by estimating (i) the delivery

of sediment through the drainage system and ii) the sediment coupling-decoupling between slopes and channels. Connectivity analysis has its roots in landscape ecology and emerged as a tool to quantify the spatial connection between different habitats. It is used to analyze the spatial relationships of these patches to understand how organisms might move and interact across a landscape (e.g. species survival, gene flow), with applications in conservation and urban planning (Luque *et al.*, 2012). In this context, a flow connectivity approach (Wainwright *et al.*, 2011) was used as a local metric to simulate mass and energy redistribution within the Greve basin, Florence, Italy. The functional connectivity metric (Wainwright *et al.*, 2011) which depends on the connectivity structure of the geomorphic and hydrologic processes was coupled to the ecologic processes by employing bioindicator microorganisms. The biotic component of the landscape was used as a new weighting factor ( $W$ ) for the basic  $IC$  calculation (eq.1) proposed by Borselli *et al.* (2008) and based on (i) the upslope component ( $D_{up}$ ) corresponding to the potential that sediment produced in the upslope contributing area and (ii) the downslope component ( $D_{dn}$ ) that characterizes the sediment potential, which is routed through the considered pixel area, has to be transported downstream to the nearest sink along the flow path.

$$IC_k = \log_{10} \left( \frac{D_{up,k}}{D_{dn,k}} \right) = \log_{10} \left( \frac{\bar{W}_k \bar{S}_k \sqrt{A_k}}{\sum_{i=k, n_k} d_i W S_i} \right) \quad [\text{eq. 1}]$$

Where:  $S$  slope gradient,  $W$  weighting factor related to the impedance to flow and sediment transport (C factor, USLE/RUSLE),  $A$  contributing area upstream of the cell, reference unit and  $d_i$  distance of the cell, reference unit from the permanent drainage network.

The spatial distribution of the F:B abundance ratio bioindicator (Pelacani *et al.*, 2025) for the Greve basin was employed as a new weighting factor ( $W$ ) for the calculation of the new connectivity index ( $CI_{FB}$ ). As a biomarker we considered the fungi to bacteria ratio as they are the main functional groups of soil organisms and are influenced by soil texture and hydrological characteristics (Djemiel *et al.*, 2023), where the soil moisture represents a critical driver for microbial activity and soil multifunctionality (Delgado-Baquerizo *et al.*, 2017). Previous studies conducted by the authors on the soil microbial composition had highlighted that the soil texture influenced the bioindicator F:B ratio, e.g. conglomerate soils showed a higher value than calcareous soils for soil fungal biomass (Roccotelli *et al.*, 2024; Pelacani *et al.*, 2025).

A grid statistics, specifically a standard deviation (std), was used to highlight the spatial patterns of CI and  $CI_{FB}$  and to analyze their environmental variation, since the std quantifies heterogeneity in the data.

### Soil erodibility and biomarkers

The spatial distribution models of the soil erodibility (K factor; USLE model) and biomarkers for the Greve basin were performed based on a few soil sampling campaigns carried out in 2018, 2021 and 2024. The soil sampling was carried out during the spring season to avoid that any chemical treatment had been done to the olive crops as it would have created a temporary alteration of the soil biological characteristics. A total of 83 samples (with replicates) were taken in a week of work from olive groves belonging to six geochemical environments of the Greve basin (fig. 1): limestone, siltstone, sandstone, shale, volcanic rocks and conglomeratic deposits. This approach allowed to sample the maximum lithological variability of the study area and from each lithology five soil samples were collected from topsoil. The soil hillslope catena concept (Ruhe and Walker, 1968) was taken into consideration for soil sampling.

The soil erodibility (K) was achieved based on the pedofunction proposed by Torri *et al.* (1997) and Torri *et al.* (2002) that consider the soil texture and the organic matter (table 1).

$$K = 0.00293(0.65 - Dg + 0.24 Dg^2) e^{(-0.0021(OM/C) - 0.00037[(OM/C)^2]4.02C + 1.72C^2)} \quad [\text{eq. 2}]$$

Where C is the clay content, OM is the organic matter content and Dg is the logarithm of the geometric mean of soil particle size.

The soil microbial abundance was determined in the samples collected in the 2021 by extracting the total genomic DNA and performing qPCR (quantitative polymerase chain reaction) to determine the 16S rRNA gene copy number of bacteria and the 18S rRNA gene copy number of fungi (Pelacani *et al.*, 2025). Furthermore, metabarcoding sequencing of the 16S rRNA gene was performed, according to the analytical procedure reported in Roccotelli *et al.* (2024) to analyze the soil bacterial composition and its taxonomic abundance.

### Mapping the soil erodibility

A digital terrain model (DTM) with a 12m spatial resolution (TanDEM-X radar imaging satellite platform) was employed for calculating the terrain indices used for the soil erodibility, sediment transport capacity index (TCI, Desmet and Govers 1995) and connectivity index spatial distribution analyses. A workflow of the analyses was reported in fig. 2. The following terrain indices were derived in SAGA GIS from the pre-processed DTM (fill sink, Wang and Liu 2006): (i) two basic terrain derivatives (slope and aspect); (ii) topographic wetness index (TWI) based on modified catchment area and slope tangent (Sørensen *et al.*, 2005); (iii) valley depth (VD); (iv) channel network base level (CNBL); (v) vertical distance to channel net-

Table 1 - Landform and texture classes data, organic matter (SOM), erodibility factor (K), Fungi to Bacteria ratio (F:B) for topsoil collected in contrasting lithologies of the Greve basin (Florence, Italy). The soil classification obtained from Tuscany region soil database was referred to the World Reference Base (WRB – FAO, 2015). The data refers to average values.

Landform	Lithology	Soil Classification WRB	Sand (2 mm)	Silt (50 µm)	Clay (2 µm)	SOC (%)	K Factor	F:B
Graded	ARENAUCEOUS MARLS	Calcaric Regosols	74.7	15.2	10.2	3.6	0.023	n.d.
	SANDSTONE (MAC)	Eutric Cambisols	76.8	15.1	8.1	0.5	0.038	5.50
Midslope ridges	FLUVIAL CONGLOMERATE (VILa)	Endoskeleti Calcaric Cambisols	52.6	27.1	20.3	2.2	0.035	7.49
	PALOMBINI SHALES (APA)	Calcaric Endoleptic Cambisols	26.4	42.4	31.2	1.6	0.036	n.d.
	MARLS (Marne di S. Polo)	Calcaric Endoleptic Cambisols	64.0	18.5	17.5	2.1	0.029	3.66
Upper slopes	SILTSTONE (PTF)	Eutri Epileptic Regosol	39.8	51.8	8.4	1.2	0.052	3.03
	SANDSTONE (MAC)	Eutric Cambisols	75.2	13.4	11.4	2.2	0.031	n.d.
	VARICOLOURED SHALES (AVR)	Calcaric Regosols	29.5	66.0	4.5	1.3	0.040	5.51
	CLAYSTONE (SIL)	Calcaric Regosols	47.2	34.5	20.2	2.7	0.039	6.47
	SERPENTINITE BRECCIAS	Calcaric Regosols	75.3	14.1	10.6	2.7	0.028	n.d.
Open slopes	BASALTS (bas)	Calcaric Endoleptic Cambisols	61.9	31.5	6.6	2.3	0.029	2.18
	LIMESTONE (MLL)	Calcaric Regosols	29.7	53.6	16.6	1.2	0.050	3.88
	BASALTS (bas)	Calcaric Endoleptic Cambisols	76.7	8.6	14.4	3.6	0.025	n.d.
	CARBONATIC FLYSH (Flysh)	Endoskeleti Calcaric Cambisols	57.6	22.4	20.0	2.8	0.028	8.45
	VARICOLOURED SHALES (AVR)	Calcaric Regosols	34.5	51.3	14.2	1.1	0.050	n.d.

work (CND) (Conrad *et al.*, 2015); (vi) Length Slope Factor (LS Factor) following Conrad *et al.* (2005); (vii) Flow path length (Multiple flow direction – FD8; Freeman 1991, Quinn *et al.*, 1991); (viii) Contributing area (Multiple flow D-infinity algorithm; Tarboton, 1997) and (ix) Landforms (Jasiewicz and Stepinski, 2013). Further, the transport capacity index (TCI, Desmet and Govers 1995), was used to evaluate the impedance of sediment transport from the hillslope to channel and total energy stored in the system. This index is directly related to the upslope contributing area and topography steepness.

To predict the spatial distribution of the soil erodibility factor and the bioindicators we applied:

(1) The Stochastic Gradient Boosting (SGB - Friedman, 2002; Salford Predictive Modeler) to predict the spatial distribution of K factor based on the sample data and combined with environmental co-variables. As input to the model, we used data from multiple sources (fig. 2): specifically, (i) Sentinel-2 satellite image: Normalized Difference Vegetation Index (NDVI) and Normalized Difference Water Index (NDWI); (ii) terrain data from the TanDEM-X radar imaging satellite platform, and (iii) rare earth elements (REEs) fractionation ratios (Lanthanum/Samarium and Lanthanum/Ytterbium) were used to consider the influence of lithological setting, since they are spatially distributed, unlike lithological classes (Pelacani *et al.*, 2024). REEs reflect the mineralogical and geochemical signatures of the rocks and indicate the geochemical evolutionary processes of the sedimentary systems (Aide and Aide, 2012). Light-REEs (LREE) are more mobile and preferentially exported by surface and subsurface runoff, while the main

control on the distribution of Heavy-REE (HREE) in the topsoils are related to bedrocks and an enrichment could be aspect in areas prone to soil erosion or intense weathering, because they are less mobile (Han *et al.*, 2025; Li *et al.*, 2020).

The SGB was used also to assess the variable importance of the terrain-geology relationship and explore the relative influence of different environmental drivers on the K factor.

(2) The spatial distribution of the bioindicator F:B was performed by using the soil erodibility factor information and the connectivity index; these variables were used as predictors in the SGB machine-learning model.

Furthermore, the genus ratio *Gaiella/Rubrobacter*, belonging to the phylum Actinobacteria, was used to predict the spatial distribution of F:B ratio. The bacterial genus *Gaiella* was found as the key functional group of bacteria for graded arenaceous soils of the Greve basin and the bacterial genus *Rubrobacter* for gentle slope soils of calcareous/carbonate lithologies (Pelacani *et al.*, 2025).

## RESULTS

### *The soil erodibility factor (K)*

The soil sampling sites cover the main lithologies of the Greve river basin (table 1; fig. 1). For the study area the K factor, a measure of how susceptible a soil is to erosion processes, ranges between 0.023 in arenaceous marls and 0.062 Mg ha h ha<sup>-1</sup> MJ<sup>-1</sup> mm<sup>-1</sup> in limestone-derived soils.

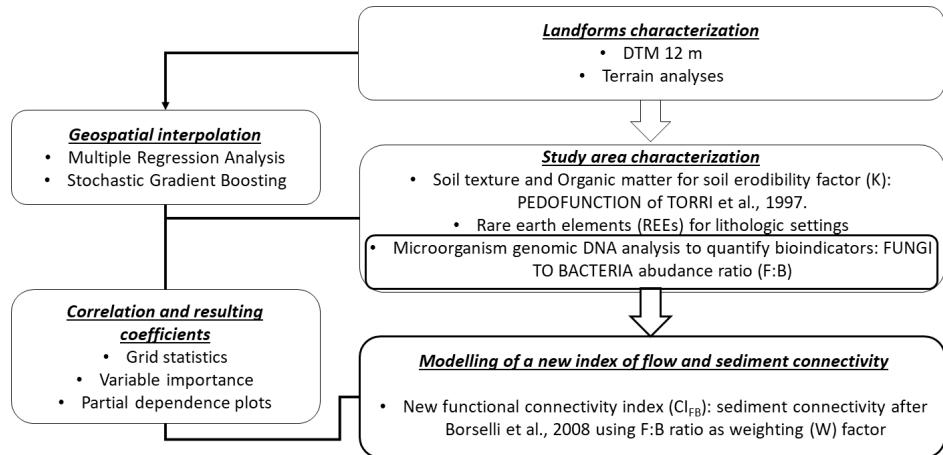


Figure 2 - Workflow for the functional connectivity assessment for the Greve basin based on bioindicators.

The values of clay and silt content in the topsoil ranged from 4.5% to 20.3% and 8.6% to 66.0%, respectively. The mean SOC value was 2.07%, ranging from 0.5 to 3.6%. The collected soil samples were dominated by silt fraction; thus, the majority of the soils were classified as silt loam or sandy loam according to USDA textural classification.

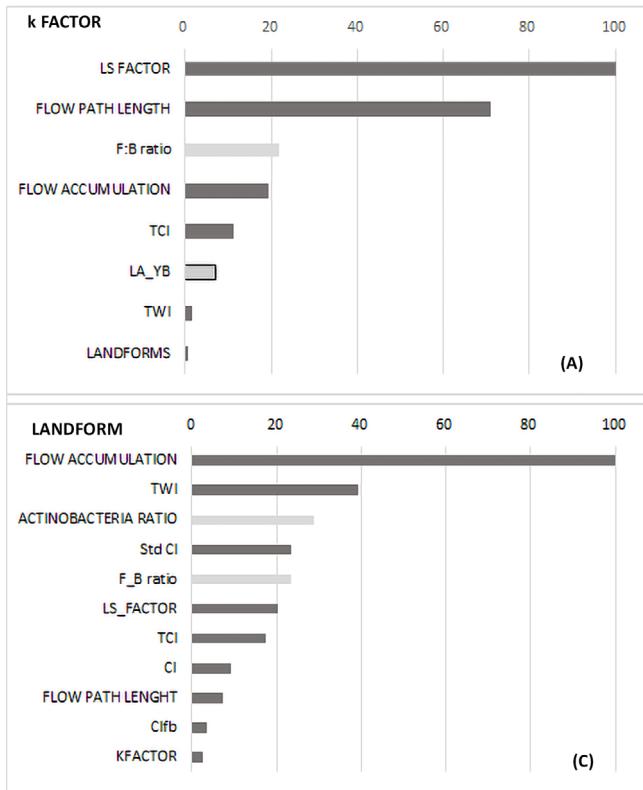
In this study ten covariates were used to model the soil erodibility factor and their relative variable importance was calculated by SGB model and reported in fig. 3. The variable importance plot provides the score that each variable contributes to predicting the response. The variable with the highest raw importance score is assigned a relative importance of 100%. SGB calculates variable importance by using the cumulative sum of the improvement scores of all the splits associated with a given variable across all trees in the ensemble (Minitab, 2019. *Introducing TreeNet® Gradient Boosting Machine*. Available online at: [https://www.minitab.com/content/dam/www/en/uploadedfiles/content/products/spm/TreeNet\\_Documentation.pdf](https://www.minitab.com/content/dam/www/en/uploadedfiles/content/products/spm/TreeNet_Documentation.pdf), last access 03 November 2025). The SGB model accuracy for the soil erodibility was equal to 99.4% ( $R^2=0.994$ ). The LS factor was the most important variables to predict the variation of the soil erodibility in the Greve watershed (fig. 3). Landform, topographic wetness index (TWI), Lanthanum/Ytterbium ratio and transport capacity index (TCI) were less important accounting for eleven percent of importance in respect to LS factor, which is the most important covariate in predicting the K factor characterizing the Greve basin.

Furthermore, the partial dependence plots (PDP, fig. 5) allow insights into the interactions of the covariates, specifically in prediction of F:B ratio. The two PDP show how K factor and CI index influences the prediction of F:B ratio (fig. 5a-b) while averaging with respect to all the other features of a machine learning model. A flat PDP with lower log odd indicates that the feature is not important, and the more the PDP varies, the more important the functionality. A linear correlation with a positive contribution for the F:B

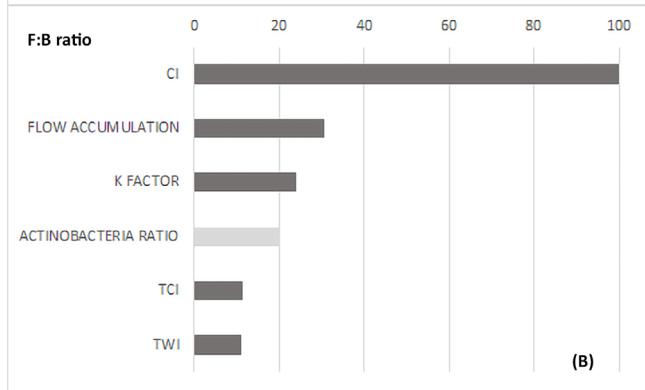
model was found at a CI index value of approximately -2.5 (fig. 5a). Considering the K factor, response curve showed linear relationship with a negative contribution between lower value of K factor ( $<0.025$ ) and the F:B ratio; however, when the K-factor exceeds 0.025, the response curve shows no further impact on the F:B model (fig. 5b). In contrast, for the K-factor model, an important functionality was found, with a step and pool pattern, for the contribution of the F:B ratio with a value greater than 6 (fig. 5d). For the F:B ratio, we observed a step-pool pattern with a positive value of the odd log ratios related to the Actinobacteria ratio values ranging from 0.05 to 0.08 (fig. 5c).

#### *The structural connectivity index-bioindicators relationship*

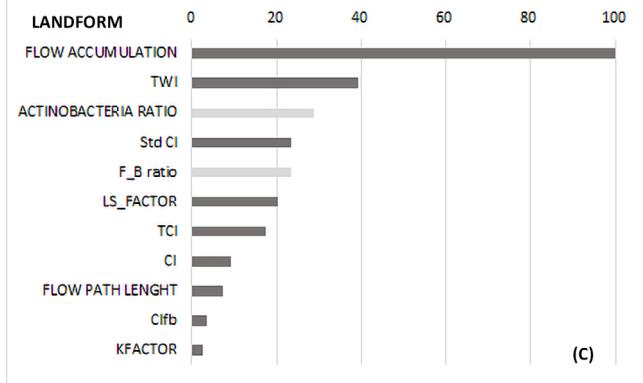
The application of a structural CI in the Greve catchment allowed the understanding of the interaction between CI and the F:B ratio. A CI was calculated according to the method proposed by Borselli et al. (2008) to define the potential connection between the runoff path from the hillslopes and the different sediment storage areas within the catchment. Furthermore, quantification of the hydro-sedimentary connectivity between hillslopes and the sinks in the Greve catchments provided information on the sediment transfer processes and their preferential pathways along the river networks. The geomorphic connectivity CI ranges between -9.0 and +0.6 (fig. 4a). Therefore, using the F:B factor, as weighting factor, we observe an increase in connectivity between landscape units, strengthening the relationships; in fact, applying the weighting factor (F:B ratio), the CI index became higher reaching the value +4.2 ( $CI_{FB}$  - fig. 4b). Highly connected systems rapidly transmit responses to disturbances throughout the system, while responses to disturbances in disconnected landscapes may be absorbed into some parts of the system. Highly disconnected valleys were found in the eastern flank of the Greve basin (figs 1d, 4e), where the change in landform along the mainstem may limit the downstream sediment flux.



(A)



(B)



(C)

The strength of connections between network elements was assessed by the quantile standard deviation, that represents the risk assessment of flow and sediment transport or deposition. The quantile 0.6796 represents the average  $CI_{FB}$  (fig. 4b) separating hillslope elements that are connected by a continuous downslope path of elements with soil moisture above a threshold value. This result was confirmed by the PDP plot of  $CI_{FB}$  that showed a positive relationship related to Landforms (fig. 5). Considering the F:B ratio, we observed a step-pool pattern with a positive value of the odd log ratios related to the Landforms for higher values of fungi to bacteria ratio. The geomorphic connectivity (CI) showed an opposite trend respect to  $CI_{FB}$ . In the downstream reaches of the Greve basin, higher  $IC_{FB}$  values ( $> +2$ ) were observed along the tributaries and main river channel (fig. 4b) compared to the upstream that showed moderate values (-1.6 to -1.1). Upper slope and open slope contribute to high IC values. The Greve basin showed high connectivity between hillslope and channels especially in the central part of the catchment; also in the left side the main river is well connected to the outlet to provide efficient sediment pathways (figs 1d, 4b). A few areas with low connectivity in the main stream were observed, that could be related to the topographic feature, alluvial deposits and check dams. In particular, areas with less consistent sediment transfer were observed at the outlet of the Ema river, the main tributary of the Greve river (fig. 1).

Figure 3 - Variable importance calculated for SGB in relation to the most important predictor for the spatial distribution of: (a) the erodibility factor (K) for the Greve watershed, Florence, Tuscany, Italy, considering terrain predictors, F:B ratio and rare earth fractionation ratio; (b) F:B ratio for topsoil and (c) Landforms. The variables are expressed in decreasing order and normalized to 100%. Legend: Fungi to bacteria ratio (F:B), transport capacity index (TCI), Lanthanum/Ytterbium (La\_Yb), Topographic Wetness Index (TWI), Standard deviation between CI and  $CI_{FB}$  grid (Std CI), geomorphic connectivity (CI) and structural connectivity with a weighting factor fungi to bacteria ratio (CIfb).

## DISCUSSION

Since our approach is to integrate an ecohydrological perspective into a geomorphic connectivity framework, considering the spatial distribution of microorganisms is essential to determine the functional role of these bioindicators in runoff and sediment generation. For that purpose, the fungi/bacteria ratio (F:B) was used as a weighting factor for the calculation of the structural connectivity index (Borselli *et al.*, 2008) instead of the land cover factor (C factor, USLE) which is generally used (Woznicki *et al.*, 2020). The distribution pattern of the F:B ratio in soil represents the result of the complex interactions between the two functional groups, Fungi and Bacteria, where the distribution factors were found mainly related to the carbon:nitrogen (C:N) ratio, soil texture, soil moisture and pH (Djemiel *et al.*, 2023; Delgado-Baquerizo *et al.*, 2017; Jiao *et al.*, 2021). Thus, the F:B ratio, from an operational point of view, represents a new useful bioindicator of soil health (Djemiel *et al.*, 2023). The F:B ratio of the Greve basin ranged from the lower value of 1.89 to the higher value of 10.64 in limestone and fluvi-conglomeratic derived soils, respectively (table 1). Keeping aware that for the spatial distribution of the F:B ratio in the Greve basin we only considered the soil samples collected in the olive groves, the F:B model that we used has an  $R^2=0.5$  (MLRA model; Pelacani *et al.*, 2025). This F:B model was used as a weighting factor for the structural connectivity index evaluation, however it has been improved by consider-

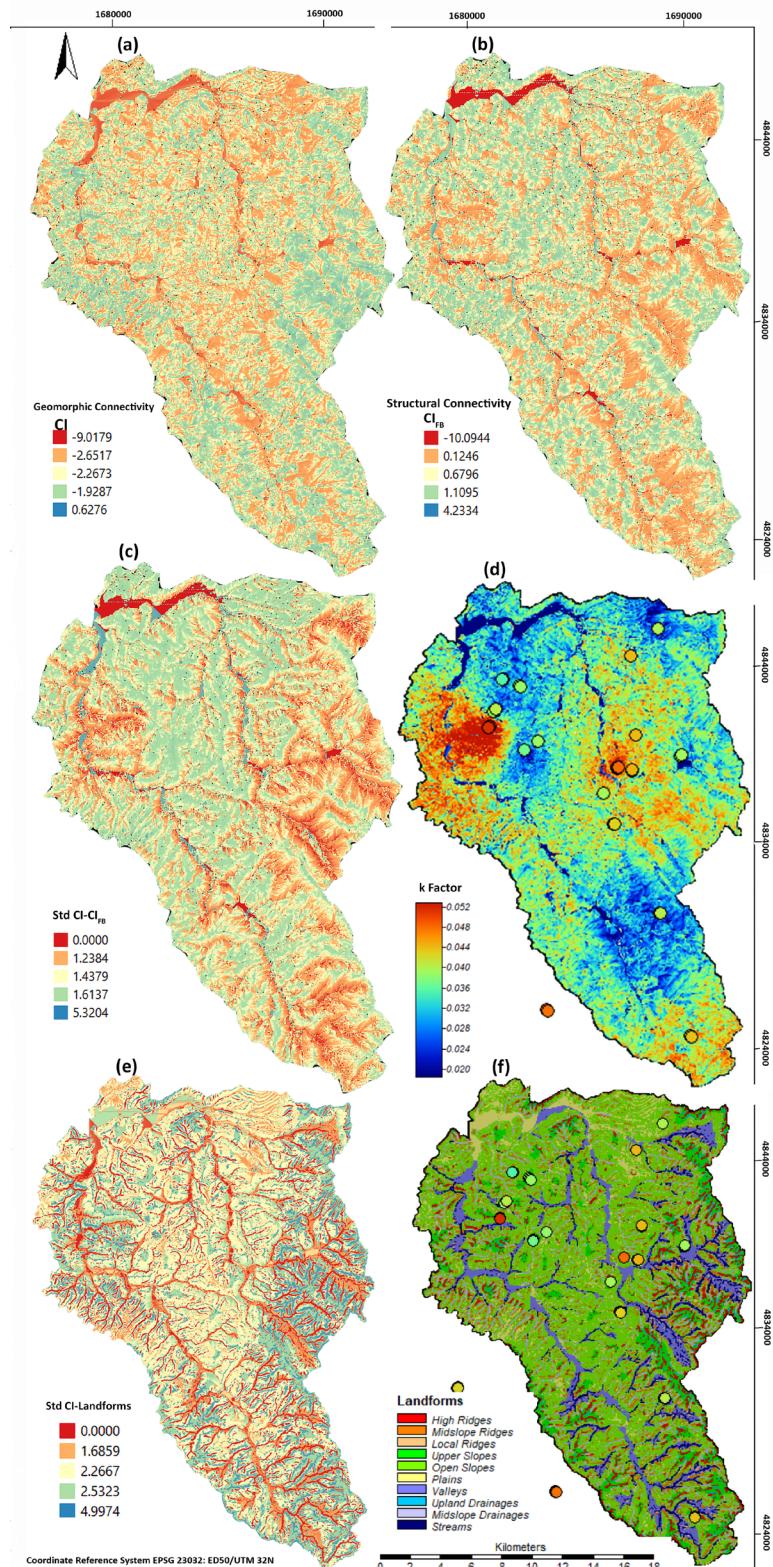


Figure 4 - The predicted map of (a) geomorphic connectivity (CI) calculated by GIS modelling approach with a 12m DTM, considering only the terrain feature (b) structural connectivity with a weighting factor fungi to bacteria ratio(CI<sub>FB</sub>), (c) standard deviation between CI and CI<sub>FB</sub> maps, (d) erodibility factor (K), (e) standard deviation between geomorphic connectivity index (CI) and landforms, (f) landforms of the Greve basin (Florence, Italy). The legend of maps (a), (b), (c) and (e) refers to quantile intervals.

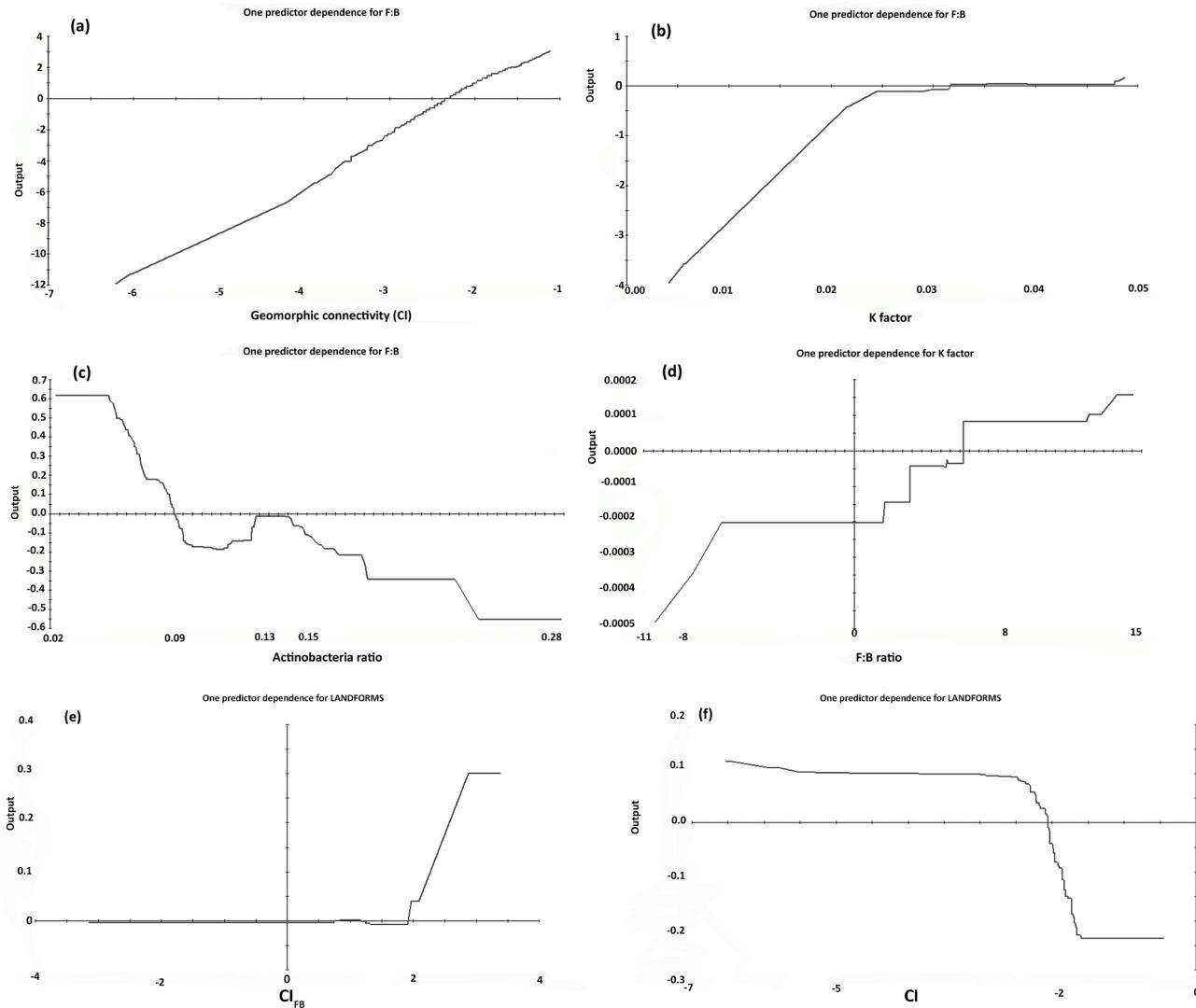


Figure 5 - Single variable partial dependence plots (PDP) for the three most important variable predictor (a) CI, (b) K factor, (c) Actinobacteria ratio of F:B ratio applying SGB model for Greve watershed and (d) F:B ratio important variable predictor for K factor. Single variable partial dependence plots (PDPs) for Landforms (e) and (f) related to Connectivity index. Partial dependence is the effect that a predictor has predicting the dependent variable, when all other predictors in the model are set to their mean value. The x-axis shows the full range of values of the predictor variable. The y-axis shows the backwards log transformation of a log-odd to produce a probability metric for a presence prediction for F:B ratio (a), (b), (c) or the K factor (d).

ing also the geomorphic connectivity and soil erodibility as independent factors, allowing to get a more accurate model (SGB model) with  $R^2 = 0.64$ .

Results showed a difference in the values of CI between the two models (CI and  $CI_{FB}$ ; fig. 4a-b). This was partly expected and agrees with previous work on the use of different W factors for the calculation of sediment connectivity (e.g. Hooke *et al.*, 2021; Martini *et al.*, 2022). This difference in the CI index values with the various W (e.g. roughness index, Manning coefficient) has been attributed to their different spatial distribution (Martini *et al.*, 2022). In the present study, a novel research work was proposed using a bioindicator for the calculation of a W factor for the sediment connectivity, that is not easily comparable to existing models.

A positive correlation was found between F:B factor and well-connected areas in the catchment ( $CI > -2.6$ ) and the  $CI$  (figs 3a, 5a). Although there are no studies regarding the relationship between geomorphic connectivity and F:B ratio, previous studies showed that a larger ratio of fungi:bacteria was related to upper slope positions compared to lower slope (Zhang *et al.*, 2013). Fig. 3 provides important information about parameter responses. The variable importance analysis likely showed a relationship because the geomorphic connectivity index, F:B ratio and erodibility factor are all interconnected through their impact on soil structure, land use, nutrient cycling, and microbial community dynamics. A healthy soil ecosystem, characterized by a balanced F:B ratio and high SOM con-

tent, is likely to have lower soil erodibility (Addesso *et al.*, 2025). Soil erosion can disrupt soil microbial communities, leading to changes in the F:B ratio. Conversely, a stable microbial community can help mitigate erosion by improving soil aggregation and providing a more stable surface. Despite F:B ratio in soil affects erodibility, impacting soil aggregation and structure (Yang *et al.*, 2023), the response curve showed major relationship between lower value of K factor (<0.025) and the F:B ratio (fig. 5b). This indicates that there is a high response of the system in correspondence with arenaceous-derived soils (table 1). A previous study conducted by the authors in the same area demonstrated that the bacterial genus *Gaiella* was the key functional group of microorganisms for graded arenaceous soils and the bacterial genus *Rubrobacter* for gentle slope soils of calcareous/carbonate lithologies (Roccotelli *et al.*, 2024). A negative relationship with higher response were found related to the F:B ratio for lower value of the *Gaiella*/*Rubrobacter* ratio (fig. 5c). Overall, these results demonstrate that the F:B ratio can be considered an intrinsic structural property of landscape units and can be used as a soil ecological bioindicator able to highlight hillslope-channel coupling situations that could not be identified with the geomorphometric approach alone. As shown in fig. 5c, the relationship between the standard deviation of a geomorphic connectivity and a structural connectivity (F:B as weighting factor) can be used as a measure of the variability of functional connectivity, particularly to assess how the strength or variability of connections (coupling/decoupling) between various parts of the catchment over different time-scale. The purpose of the model developed in this paper was to help us to better understand how topography and hydrological dynamics coevolve with the microorganisms. The variability in sediment connectivity was much greater along the river channels (e.g. in the longitudinal connectivity) when using the new index  $CI_{FB}$  compared to the standard sediment connectivity index (Borselli *et al.*, 2008), likely due to the new index being more sensitive to localized changes in topography. This significant difference in standard deviation ( $std = 5.3$ ; fig. 4c) means that the new index captures finer-scale spatial heterogeneity in connectivity. The new index also shows areas of very high and very low connectivity coexisting close together along the river channels (fig. 4b), which the standard index CI had not been able to differentiate so clearly (fig. 4a). By capturing more spatial variation details, the new index  $CI_{FB}$ , hence, can provide a more accurate assessment of the sediment transport and deposition processes, which is crucial for managing erosion control, landslide risk assessment, and geomorphic processes. A low standard deviation ( $Std < 1.2$ ; fig. 4c) between the CI and a new  $CI_{FB}$  indicates that the two methods produced similar results, and this similarity in the east-western sector of the basin was linked to morphotectonic activity (Cornamusini *et al.*,

2011; Benvenuti *et al.*, 2014). This suggests that morphotectonic forces have influenced the sediment transport and storage in the Greve watershed, causing both connectivity models to reflect a similar pattern of landscape response. The CI scenario of the eastern flank of the Greve basin, suggests disconnected or partially connected open slope, mostly affected by a quiescent landslides. This geomorphic dis-connectivity may impact the stream longitudinal connectivity leading to increase in-channel erosional processes downstream. Longitudinal higher connectivity ( $>1.6$ ) was predicted along the main steam of the Ema tributary (fig. 3b). Furthermore, in the study catchment, based on an extensive field survey, sediment availability is mainly driven by channel bank failures or river incision into calcareous bedrock (Monte Morello Formation). After heavy storms, some parts of the channel are affected by rapid sediment recharge, those parts being directly connected to drainages ditches or roads; as field investigations show, it is possible to define this part of the catchment as a “transport-limited channel”. The channel profile is controlled by sediment flux but at the same time the presence of fallen trees favors the deposition of blocks (also shrub layer) which over time leads to the migration of the flow from the main channel favoring wetland area ( $CI = -1.2$ ) (fig. 6). The  $CI_{FB}$  spatial distribution model could be a useful tool to identify areas that potentially influence bacterial community coalescence drivers (Qui *et al.*, 2021; Wang *et al.*, 2023). Although a bacterial community monitoring campaign related to river areas was not considered in this research, the study of the community coalescence should be considered in the application of river ecological restoration measures.

## CONCLUSIONS

In this research, a new weighting factor (W) for the sediment connectivity index based on the bioindicator Fungi to Bacteria ratio abundance (F:B) was used. Results showed that combining the geomorphic connectivity approach with the F:B ratio bioindicator can provide a more comprehensive understanding of the hydrologic connectivity degree within the watershed. Specifically, for the Greve watershed the new index  $CI_{FB}$  captures finer-scale spatial heterogeneity in connectivity respect to the CI.

The  $CI_{FB}$  model uses a measurable bioindicator, such as the ratio of fungal to bacterial biomass (F:B), to assess soil health. This approach allows for the identification of ecosystem thresholds, indicating the point where the soil's ability to recover from disturbances, is compromised. To become fully operational, the  $CI_{FB}$  model needs more data and testing to account for the diverse impacts of different land uses on processes like water management, erosion, and the environment. By using

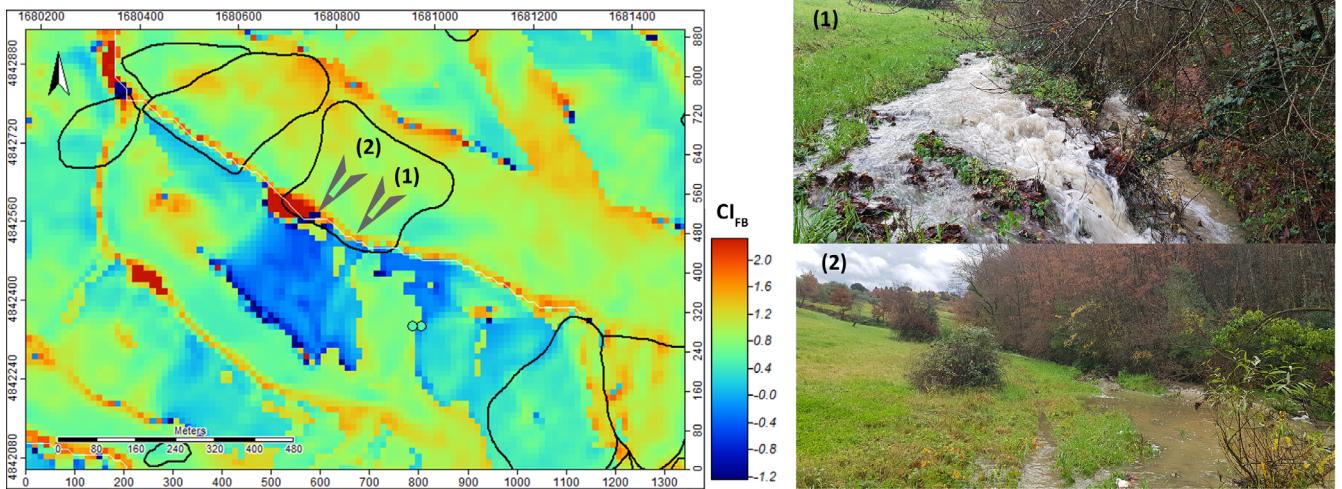


Figure 6 - Hydrological connectivity pattern for a first-order stream basin, where flow migration is driven by fallen trees. Over time, this depositional process has altered the flow path of the channel and ultimately caused wetland areas (N.2; CI = -1.2). The increased connectivity can be evidenced by the alteration of the flow path from a single channel to a more distributed network (N.1), leading to lateral bank erosion.

F:B ratio as a bioindicator, the model provides a mean to evaluate the effectiveness of different management practices in maintaining soil health and functionality. On the other hand, this approach based on connectivity index can inform on landslide assessment risk for geomorphologic threshold also in Mediterranean mountainous area. Connectivity indices quantify the flow and transport of sediment and water through landscapes, which is crucial for understanding geomorphological processes and their potential to trigger hazards. The results of this research could be useful for future studies exploring the intricate relationships between environmental factors, such as topography, vegetation and soil processes, and the resulting ecosystem functions on hillslopes.

#### AUTHORS' CONTRIBUTION

Samuel Pelacani: Conceptualization, Methodology, Data analysis, Original draft preparation and editing, funding acquisition; Federico Raspini: Methodology; Review and editing; Angela Roccotelli: Methodology; Data analysis; Review and editing; Francesco Barbadori: Investigation; Review and editing; Maria T. Ceccherini: Methodology; Review and editing; Simone Tommasini: Methodology; Review and editing; Sandro Moretti: Review and editing; Supervision & funding acquisition.

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