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Influence of pyroclastic soil on epikarst formation: a test study in southern Italy

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ABSTRACT

Epikarst formation in the southern Apennines (Italy) was hypothesized to be significantly influenced by diffuse rainwater infiltration through soil of pyroclastic origin. Multidisciplinary investigations were carried out at an experimental field site to test this hypothesis. At this site, other factors influencing epikarst formation can be assumed invariant. A direct relationship was observed between soil thickness and epikarst thickness. This relationship supports the hypothesis that the pyroclastic soil plays a significant role in governing epikarst evolution and thickening. The sandy loam texture and the high hydraulic conductivity of the soil allow easy rainwater infiltration, therefore causing a diffuse interaction between percolation water and the soil medium, and spatially homogeneous recharge within the aquifer system. The soil contains a large accumulation of organic carbon and considerable CO_2 production results from the activity of autochthonous microorganisms belonging to different genera.

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Introduction

The epikarst is the uppermost zone of carbonate rocks in which permeability, as a result of fissuring and diffuse karstification, is substantially greater than in the underlying rock mass (Klimchouk, 1997). The contrast in permeability between the epikarst and the underlying fractured bedrock influences whether water storage in a temporary perched aquifer takes place (Mangin, 1975) or not (Petrella *et al.*, 2007). The existence distribution and hydrogeological behaviour of epikarstic zones may significantly influence the recharge function of carbonate aquifers at a basin scale (Aquilina *et al.*, 2005), as well as their vulnerability to pollution (Dörfliger *et al.*, 1999). Thus, this information is needed to implement a thorough hydrogeological model of carbonate aquifers.

Epikarst formation in the southern Apennines (Italy) was hypothesized to be significantly influenced by diffuse rainwater infiltration through soil of pyroclastic origin (Petrella *et al.*, 2007). The aim of this study was to investigate this hypothesis and also to analyse some soil factors that may influence carbonate dissolution and then epikarst formation. Thus, the study was carried out in a relatively small area where other important factors influencing epikarst formation (parent rock, tectonic structure and regime, climate and microclimate; Klimchouk, 2004) can be assumed invariant. The results of such a study will have important implications in forecasting the existence and main features of epikarstic horizons in carbonate aquifers with pyroclastic covers.

Study area

The Acqua dei Faggi experimental site (southern Italy; Fig. 1) mainly consists of limestone, and subordinately of marly limestone and marls. Carbonates lie below a soil of pyroclastic

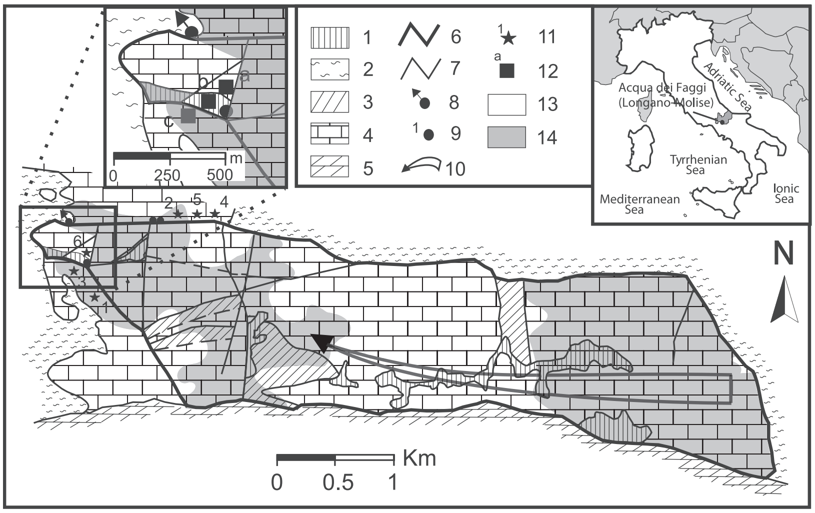

Fig. 1 Hydrogeological map (1, quaternary deposits and karstic depression; 2, marls and clays; 3, marls and marly limestone; 4, limestone; 5, dolostone; 6, aquifer boundary; 7, fault; 8, perennial spring; 9, seasonal spring; 10, main groundwater flow direction; 11, VES; 12, soil sampling sites in (a) woodland, in (b) the karstic depression and (c) grassland; 13, woodland; 14, grassland).

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origin (Celico *et al.*, 2004; Naclerio *et al.*, 2008) classified as Vitric Andosol (FAO, 1988). The soil is up to several metres thick in small karstic depressions, up to 1.5 m thick in woodland slopes and up to a few tens of centimetres in grassland slopes (Fig. 2).

The aquifer is bounded by low-permeability rocks and/or faults, which have produced significant cataclastic zones. Within the aquifer, the lower permeability of some fault cores causes the aquifer to be compartmentalized, although significant groundwater flowthrough was observed. Several seasonal (Celico *et al.*, 2006) and temporary (Petrella *et al.*, 2009a) springs occur along some low-permeability fault zones. At a basin scale, the groundwater flows westwards towards the perennial spring (Fig. 1).

The continuum approach can be applied to describe groundwater flow at the metre scale (Petrella *et al.*, 2007).

At this site, the epikarst was thoroughly investigated in a karstic depression (Petrella *et al.*, 2007, 2008), where it is made up of two horizons. Darcy's law can be applied in the lower epikarst. Groundwater flow is expected also to be laminar in the upper epikarst and in the underlying fractured bedrock (Petrella *et al.*, 2008).

The permeability contrast at the base of the epikarst is not large enough to cause significant retention of percolation and water storage in a temporary perched aquifer during rainwater infiltration.

Fig. 2 Examples of pyroclastic soil in woodland (top), and in grassland (bottom).

Methodology

Geoelectrical survey

Six Schlumberger vertical electric soundings (VES; Fig. 1) were performed on those hills with sub-planar tops that are sufficiently extended to reconstruct the shallow electrostratigraphy of the areas. Since the epikarst in a karstic depression was detected and characterized (Petrella *et al.*, 2007, 2008), the soundings were carried out within grassland (VES 1, 2 and 3) and woodland (VES 4, 5 and 6). The soundings were modelled using a simple 1-D terrain structure. As these hills with sub-planar tops are elongated in one direction, it was not possible to attempt 2-D profiling. However, using the model obtained by Petrella *et al.* (2007) for computing the expected resistivity curves, an *a posteriori* check of the validity of the suggested models was performed.

Geophysical methods, and especially the VES method, have been successfully used in groundwater exploration (e.g. Koefoed, 1979; Goldman and Neubauer, 2004), since they are usually non-invasive and relatively cheap.

Soil characterization

Thirty-two soil samples were collected randomly from the test site. To minimize disturbance of the samples, grass-covered blocks (181.36 cm square by 11 cm deep) were carved using the method described in Celico *et al.* (2004) and Naclerio *et al.* (2008). Hydraulic conductivity of soil blocks was measured by means of a standard permeameter. After these measurements were made, grain size and organic matter content were analysed according to ASTM standard procedures.

Enumeration of soil bacteria and identification of CO_2-producing bacteria in soil

Soil samples were collected (Fig. 1) (a) from the karst depression where Petrella *et al.* (2007, 2008) had thoroughly characterized the epikarst and (b) from two of the sites where VES were performed in grassland and woodland, to determine the soil bacterial numbers and to detect the presence of CO_2-producing bacteria in soil.

For enumeration of soil bacteria, soil samples were collected at three different depths (3, 10 and 50 cm below ground surface) in the karstic depression and in woodland, and at two depths (3 and 10 cm below ground surface) in grassland. Soil bacterial counts were determined through the method described by Egdell *et al.* (1960).

To detect CO_2-producing bacteria in soil, biomolecular investigations were carried out using soil samples collected (a few cm below ground surface) at each of the sites described above. Nucleic acid extraction from soil samples was carried out using the PowerSoil^{TM} DNA Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Biomolecular investigations (PCR-DGGE and 16S rDNA sequencing) were carried out as described in Petrella *et al.* (2009b).

To detect and classify CO_2-producing *Bacillus* strains, methods to identify individual species were applied (Naclerio *et al.*, 2009a).

Investigation of carbonic anhydrase enzyme in soil

Three soil samples were collected from each of the sites described in the previous paragraph, to analyse the carbonic anhydrase (CA) activity in the soil. CA is a Zn-containing metalloenzyme that catalyses the reversible hydration of CO_2 ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) (Lindskog *et al.*, 1971). It has been demonstrated that CA may significantly enhance the dissolution rates of limestone (Liu and Dreybrodt, 1997) and then karstification (Li *et al.*, 2005). The activity of CA in soil was determined by the method described in Li *et al.* (2005).

Buffering capacity of soil

A physical analogue model was developed through column experiments to analyse the buffering capacity of the soil. Three intact soil blocks ($181.36\ cm^2$ by 11 cm deep) were collected from the test site according to

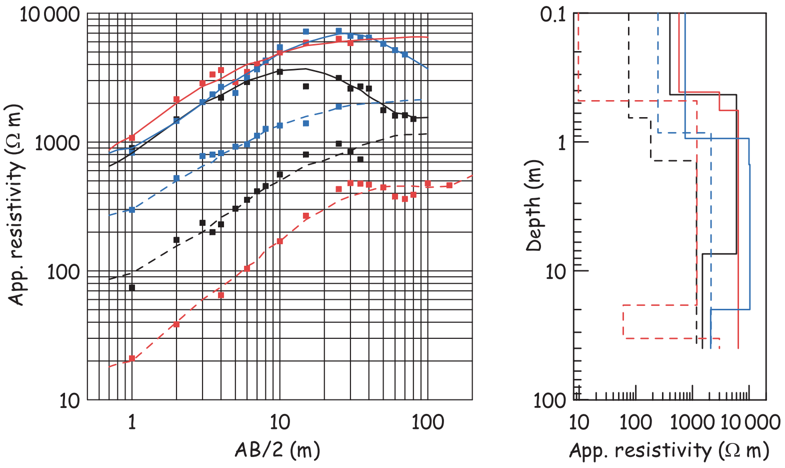


Fig. 3 Apparent resistivity vs. AB/2 (squares represent the experimental data and the continuous line represents the model data). 1-D models are also reported (full black line is VES 1, full red line is VES 2, full blue line is VES 3, dashed black line is VES 4, dashed red line is VES 5, dashed blue line is VES 6).

the method described by Celico *et al.* (2004) and Naclerio *et al.* (2008). A diffuse water flow (3600 mL of deionized water) was obtained by carrying out the column experiments in a standard permeameter. Three experiments were carried out, each one at a different solution pH (3.0, 5.6 and 9.0).

Results

Geoelectrical survey

Vertical electric soundings 1, 2 and 3 (Fig. 3) were characterized by 0.4–0.9 m of limestone with electrical resistivity (ρ) ranging from 400 to 750 Ωm, below which the resistivity abruptly rises to 6000–10 000 Ωm. VES 1 and 3 showed a decrease in ρ below the higher resistivity horizon, probably because of interbedded marly limestone and marls. These VES did not detect the soil cover because of its thinness. In contrast, VES 4, 5 and 6 (Fig. 3) detected a lower resistivity (10–250 Ωm) soil (0.5–1.5 m deep) that lies above a limestone bedrock with $\rho \cong 1200$–2100 Ωm (VES 4 and 6) and $\rho \cong 800$ Ωm (VES 5).

In the karstic depression, previous geoelectrical investigations suggested the following scenario (Petrella *et al.*, 2007). The soil cover is about 5-m thick and is characterized by $\rho = 33$ Ωm. The soil overlies an upper 5-m-thick epikarstic horizon (ep1), diffusely karstified, with $\rho = 465$ Ωm, that lies above a lower 5-m-thick epikarstic horizon (ep2), characterized by non-pervasive karstification, with ρ ranging from 1200 to 1500 Ωm. The deeper limestone bedrock, characterized by a poorly developed discontinuity network, is characterized by a higher resistivity value ($\rho \cong 10\ 000$ Ωm; Petrella *et al.*, 2009b), in agreement with the findings at other sites (e.g. Stepišnik and Mihevc, 2008). The physical model developed for the Acqua dei Faggi experimental site was obtained by additionally using borehole stratigraphies and continuous hydraulic head measurements and performing Lugeon tests and tracer tests.

Taking into consideration the previous studies, the results obtained in woodland and grassland suggest significant differences in terms of fracturing and karstification of carbonate rocks. In more detail, the following scenario can be depicted. Where a thin soil lies above limestone (grassland), the near-surface carbonate bedrock (up to 0.9 m) is dominated by wider conduits. This epikarst type lies directly above a fractured bedrock. Where a thicker pyroclastic soil lies above the carbonate rocks (woodland), the VES clearly detected epikarstic horizons with pervasive (VES 5) or non-pervasive (VES 4 and 6) karstification. Unfortunately, the maximum electrode distances did not allow determination of the exact thickness of such horizons, but they are at least several metres thick.

Main features of soil

Grain-size analyses show a global homogeneity of soil samples, testified to by a narrow envelope of grain-size curves (Fig. 4) and by a prevailing sandy loam texture. Laboratory tests revealed a very high content of organic matter ranging from 24% to 34%.

According to previous results (Naclerio *et al.*, 2009a,b), the hydraulic conductivity of soil samples ranged between 4.4×10^{-6} and 8.6×10^{-5} m s^{-1} with a mean value of 2.2×10^{-5} m s^{-1} (SD 1.8×10^{-5}), therefore showing that this soil allows diffuse and easy rainwater infiltration and percolation towards the underlying carbonate bedrock.

Viable count of soil bacteria and CO$_2$-producing bacteria in soil

Counts of viable soil bacteria suggest no important differences in concentration within each soil profile (Table 1). A slight difference was observed between soil profiles, with viable counts being slightly higher in the karstic depression.

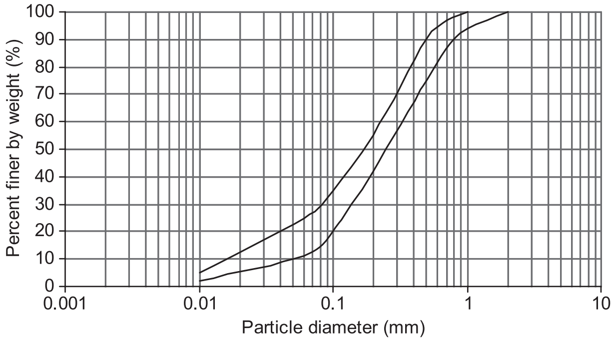


Fig. 4 Grain-size distribution range for soil samples.

Table 1 Counts of viable soil bacteria (CFU g^{-1} dry soil) at different depths below ground level (bg).

Grassland	Grassland	Woodland	Woodland	Woodland	Depression	Depression	Depression
3 cm bg	10 cm bg	3 cm bg	10 cm bg	50 cm bg	3 cm bg	10 cm bg	50 cm bg
4.1×10^5	1.2×10^6	7.4×10^5	7.4×10^5	5.3×10^5	2.9×10^6	2.6×10^6	4.7×10^6

The bacterial 16S rDNA partial sequences obtained from the DGGE gel were found to correspond with sequences listed in GenBank. On the whole, significant differences were observed between communities, probably because of different land uses. Ten sequences are similar to bacterium clones the metabolism of which is unknown; therefore, no information is available on possible CO_2 production. In woodland, strains belonging to the genera *Rhodoplanes* and *Sphingomonas* were detected, both CO_2-producing (Garrity, 2005). In the karstic depression, CO_2-producing strains (Garrity, 2005), belonging to the genera *Arcobacter* and *Clostridium*, were detected. Conversely, the strain belonging to the class *Anaerolineae*, detected in the karstic depression, does not produce CO_2 (Sekiguchi *et al.*, 2003). Investigations to identify individual species allowed the detection of strains belonging to the genus *Bacillus* in all soil samples. These strains were identified as *B. subtilis*, *B. cereus*, *B. pumilus*, *B. megaterium*, *B. amyloliquefaciens* and *B. macroides*. Carbon dioxide-producing strains detected in the studied soil produce CO_2 through different metabolic pathways.

Carbonic anhydrase enzyme in soil

Carbonic anhydrase was analysed in soil samples collected within a karstic depression, woodland and grassland. However, no activity was detected in any of the analysed soil samples. The concentration of such an enzyme in the studied soil can be below the detection limit because of one or more factors, such as:

- Carbonic anhydrase enzyme is indeed widespread and nearly ubiquitous among metabolically and phylogenetically diverse bacteria (Smith *et al.*, 1999), and plays a major role in bacterial physiology (Smith and Ferry, 2000); however, it is known that CAs from bacteria are generally of very low abundance (Smith and Ferry, 2000).
- The concentration of bacterial cells (4.1×10^5–4.7×10^6 CFU g^{-1} dry soil) in the studied soil is lower than that (e.g. 10^7 CFU g^{-1} dry soil in average, Li *et al.*, 2004) observed in other media where CA activity has been detected.
- The state of association of CA is markedly affected by bicarbonate concentration. The enzyme exists as a tetramer in the absence of added bicarbonate, but as a dimer in the presence of added bicarbonate (5 mM; Guilloton *et al.*, 1992). Perhaps the latter state of association is more susceptible to proteolysis. Thus, degradation of CA may be induced by relatively high levels of bicarbonate in bacterial cells (Kozliak *et al.*, 1994). These findings are of great importance when considering the high carbonate content of the studied soil (data not shown).

Buffering capacity of soil

The pH of water samples collected at the soil bottom during the column tests varied slightly between experiments (pH 6.9–7.3; Table 2). These results suggest a high buffering capacity of the soil, in accordance with findings in other soils developed in volcanic ejecta (e.g. Ugolini and Dahlgren, 2002).

Discussion

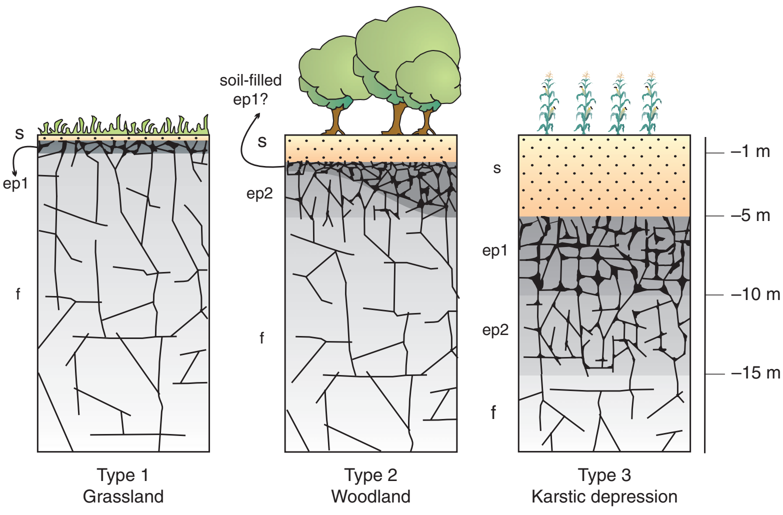
The epikarst is widely distributed within the experimental site. Three different types of pedogeological settings were recognized (Fig. 5). Type 1 is found in grassland and consists of thin (up to a few tens of cm) pyroclastic soil overlying thinner epikarstic horizons (up to 0.9 m thick) with wider conduits and very high porosity (ep1). Type 2 is found in woodland and consists of pyroclastic soil (up to 1.5 m thick) overlying epikarst with pervasive (ep1) or non-pervasive karstification (ep2). In such a setting, the whole epikarst is at least several metres thick, but ep1 is sometimes absent or, more probably, it is a soil-filled thin horizon not detectable through geoelectrical investigations. Type 3 is found in karstic depressions (Petrella *et al.*, 2007) and consists of thicker (about 5-m thick) pyroclastic soil and thicker epikarstic horizons (about 10-m thick) with both pervasive (in the upper part; ep1) and non-pervasive (in the lower part; ep2) karstification.

Table 2 pH of simulated rainwater (pH_in) and mean pH of water collected at the outflowing hole during the column experiments (standard deviation given in brackets).

Sample	pH_in	pH_out
1	3.0	6.9 (0.1)
2	5.6	7.3 (0.1)
3	9.0	7.1 (0.2)

The direct relationship between soil thickness and epikarst thickness at this site, where other factors influencing epikarst formation can be assumed invariant, supports the hypothesis that the pyroclastic soil plays a significant role in governing epikarst evolution and thickening. The sandy loam texture and the high hydraulic conductivity of the soil allow easy and diffuse rainwater infiltration. This process causes a diffuse interaction between percolation water and the soil medium, and a spatially homogeneous recharge within the aquifer system. The soil contains a large accumulation of organic carbon and considerable CO_2 production results from the activity of autochthonous microorganisms, such as species belonging to the genera *Bacillus, Rhodoplanes*, *Sphingomonas*, *Arcobacter* and *Clostridium*. Conversely, no CA activity was detected. Owing to the high buffering capacity of the soil, karstification processes are slightly influenced by variations in rainwater pH over time.

The direct relationship between soil thickness and epikarst thickness may be explained as follows. In deep soil, organic carbon concentrations in subsurface horizons (below 25 cm, *sensu* Fierer *et al.*, 2003) are generally



Type 1
Grassland

Type 2
Woodland

Type 3
Karstic depression

Fig. 5 Main pedogeological settings (s is pyroclastic soil, ep1 is epikarst with pervasive karstification, ep2 is epikarst with non-pervasive karstification, f is fractured limestone).

much lower than in surface horizons (0–25 cm, *sensu* Fierer *et al.*, 2003). Nevertheless, the total volume of soil contained in subsurface horizons can be very large. Thus, according to Batjes' (1996) findings, more than 50% of the organic carbon contained within deep soil profiles, such as the soils of karstic depressions at the study site, is found in subsurface horizons. Subsurface organic carbon pools are characterized by long mean residence times in the soil, because of a lower microbial mineralization rate (Desjardins *et al.*, 1994). Hence, although subsurface organic carbon pools are not mineralized at high rates, the amount of CO_2 produced in deeper soil horizons by microbial metabolism can be substantial and, in some soils, may account for a significant portion of total soil CO_2 production (Wood *et al.*, 1993).

As stated above, soil thickness is also correlated with different vegetation types at the study site. However, although site vegetation is an important determinant of soil CO_2 production, no predictable differences in soil CO_2 production have been found between cropped and vegetation-free soils, between forested and cropped soils, or between grassland and cropped soils when isolating the effects of vegetation type alone (Raich and Tufekcioglu, 2000). In contrast, the spreading of a huge volume of manure for agricultural purposes is expected to induce prevailing, microbial oxidation processes, with an increase in CO_2 production in karstic depressions.

Soil thickness was indeed influenced by morphological features, because the accumulation of pyroclastics was enhanced in karstic depressions, while soil erosion was minimized. In contrast, epikarst evolution is not expected to be influenced by concentrated infiltration of runoff in karstic depressions, because runoff is negligible at the study site, because of the high permeability of outcropping rocks. A role in epikarst evolution within some karstic depressions was played by groundwater flow, because great hydraulic-head fluctuations cause the groundwater to flow in the near-surface carbonate bedrock during very high flow periods.

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