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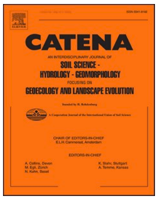

The beneficial use of basalt flour combined to a microbial consortium to improve soil quality in basalt and carbonatic dismissed quarries

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A B S T R A C T

The recovery of degraded soils to restore their capacity to carry out important ecological functions such as promoting nutrient cycling is a very topical issue. The aim of the present work was to assess soil quality changes within two different disused quarries in central Italy (named Cornale and Poggio Tabor) through the addition of basalt flour combined with a microbial consortium (BBF) (2.7 kg of basalt flour and 54 ml of microbial consortium inoculum at each supply date). Soil samples were collected within 9 plots where three treatments 1) Control (C) no treatment; 2) bioactive basalt flour (BBF) and 3) bioactive basalt flour and cover crop (BBF + CC) were randomly replicated three times per quarry. Chemical indicators showed little changes due to the different amendments, conversely, the biochemical ones showed that the addition of BBF increased enzymatic activities involved in C, N, P, and S cycling, and promoted microbial growth and C mineralization activity at both studied sites. At the Cornale site in summer, the rooting of the cover crop, through the rhizodeposition provided the carbon substrates necessary to fuel the added microorganisms showing an increase in the microbial quotient of about 5 % compared to the control soil. Conversely, at the Poggio Tabor site, the peculiar geomorphology prevented the cover crop growth; therefore, the positive responses of enzymatic activities were evident only in the BBF treated plots.

From a management perspective, the use of bioactive basalt flour consisting of basalt flour and microbial consortium could be a good strategy to restore soil quality according to nutrient cycling function in a degraded area. However, in highly limited organic matter conditions, an external input of organic carbon is strongly recommended to sustain the exogenous microbial consortium.

1. Introduction

Approximately 33 % of the Earth's soils are already degraded and over 90 % could become degraded by 2050 (IPBES, 2018; FAO, 2019). Food and Agriculture Organization of the United Nations and the Intergovernmental Technical Panel on Soils Erosion have identified organic matter loss, nutrient imbalance, acidification, contamination, waterlogging, compaction, sealing, salinization, and loss of biodiversity as the ten major threats to soil quality and responsible for degradation processes (FAO and ITPS, 2015). Being soil non-renewable resources, the likely impact of degradation on future soil capacity to carry out their many ecological functions and support human needs is of major concern within the scientific community.

Among the many factors leading to soil degradation, mining and/or quarrying activities have been widely reported to increase contamination (Ferreira et al., 2022), erosion (Sheoran et al., 2010), and alteration of soil reaction (Fu et al., 2011). Moreover, these activities can cause land subsidence and alteration of soil fertility and ecology (Ma et al., 2019), accumulation of mine dumping with modification of soil profile, and loss of organic matter.

Reclamation is the process by which highly degraded lands are returned to productivity, through the restoration of biotic functions and productivity. In particular, for this purpose, the strategies mainly adopted are the addition of topsoil, different types of amendment, and afforestation or plantation (Sheoran et al., 2010).

Among them, the use of silicate rock powders (SRP) has been recently shown to offer several benefits in restoring degraded soil nutrients pool and thus fertility (Swoboda et al., 2022).

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Amending soils with ground rocks is an ancient practice maintained in modern agriculture practices such as the addition of carbonates and gypsum for pH control and phosphate rocks as P fertilizers (van Straaten, 2006). The application of silicate rock powder has resulted in the improvement of the chemical characteristics in various types of soil such as high efficiency for neutralization of the potential acidity (Melo et al., 2012) and an increase in the availability of alkaline cations (Silva et al., 2012).

Among SRPs, basalts are among the most studied rocks because they provide nutrients for plants (Anda et al., 2015; Ramos et al., 2022). Some authors reported that in highly weathered soils of tropical agricultural regions the addition of basalt leads to an increase in crop yields, soil pH and cation exchange capacity (Van Straaten, 2006; Hartmann et al., 2013; Edwards et al., 2017; Beerling et al., 2020). Additionally, soils produced from basaltic quarry have inorganic carbon sequestration potential via in situ carbonate formation, especially when mixed with organic matter (Manning and Renforth, 2013).

Other than the addition of organic amendment (Soria et al., 2022), the microbial inoculum is also widely used as an agronomic practice aimed at improving soil quality in degraded soils. Microbial inoculum is a natural fertilizer or bio-fertilizer containing a specific population of microorganisms (bacteria and/or fungi) or a selected group of beneficial microorganisms to be supplied to the soils, thus improving their biological fertility (Rashid et al., 2016; Okur, 2018). The use of microbial inoculum could be enclosed within bioaugmentation practices capable of supporting native microbial species for remediation in a contaminated site (Baskaran and Sathiavelu, 2022).

Also, the combined effect of powdered rocks and a microbial inoculum has been proven to be efficient in mitigating soil degradation (e.g. N fixation and, P mobilization) and contributing at the same time to the immobilization of soil pollutants (Gindri Ramos et al., 2022). In general, the beneficial contribution of the added microorganisms may be observed either in the chemical and biological or the physical properties of soils (Rashid et al., 2016; Suhag, 2016). Ribeiro et al. (2020) report how rhizosphere processes combined with biological weathering (rock fragmentation induced by bacteria) may further enhance mineral dissolution since the interaction between minerals, plants, and bacteria results in the release of macro- and micronutrients that ameliorate soil nutrient status.

In the present paper, a comprehensive approach has been adopted to test basalt flour with microbial consortium treatment to amend soils of two dismissed quarries in central Italy with an attempt to recover soil quality. The assessment of the soil quality of these degraded quarry soils was obtained by integrating different categories of soil indicators (Moscatelli et al., 2015; Moscatelli et al., 2017; Bünemann et al., 2018; Moscatelli et al., 2022). The choice of the proper indicators, in particular those reflecting the chemical and biological attributes, was done taking into account soil properties that could be impacted by the amendments with basalt flour and microorganisms.

The general aim of this research was to evaluate the efficacy of basalt flour combined with the microbial consortium as a soil amendment to be used in the restoration of degraded quarry soils. The study focused on soil quality improvement according to nutrient cycling function after two soil treatments: (basalt flour + microbial consortium, henceforth named bioactive basalt flour (BBF) and basalt flour + microbial consortium + cover crop, henceforth named BBF + CC) in two dismissed quarries.

We hypothesised that the biological properties (bioindicators) may represent sensitive indicators of soil changes, able to provide prompt responses to soil treatments in a very poor nutrient background. Conversely, other soil attributes such as organic matter content, C/N ratio generally needed a much longer period to highlight significant changes in soil *quality improvement* (Bünemann et al., 2018). In this case, study we hypothesised a positive effect of BBF + CC treatment on soil nutrient cycling in a short-term period through basalt flour plant and rhizospheric activity as a source of available carbon forms triggering both microbial consortium and the native soil microflora.

According to the general hypothesis, the two specific objectives of this investigation were: i) to establish whether BBF and BBF + CC trigger soil biochemical indicators, in particular nutrient cycling, such as enzymatic activities, basal respiration, and microbial biomass carbon, and ii) to establish whether BBF and BBF + CC exert changes on soil chemical properties such as pH, cation exchange capacity (CEC), available P and exchangeable cations.

2. Materials and methods

2.1. Site description

The two sites under study are in central Italy and are managed by Basalti Orvieto srl. The first one, an area of about 800 m^2, is located at Cornale (Terni province) at 500 m above sea level (a.s.l.), the basalt extraction activity was interrupted in the period 2017 (Fig. 2). The second site, named Poggio Tabor, is located at Ficulle (Terni province) ca. 20 km far from Cornale and is an area of about 1200 m^2 at 600 m a.s.l. It was a former limestone quarry, dismissed in 2010, used to extract sandy conglomerates and is characterised by extensive rocks outcrop (Fig. 1). The climatic data (average temperature and rainfall) of the area under study relative to the 2020–2021 years were reported in Fig. 1. At all sites, the plant vegetation was spontaneous and not homogeneous, mainly herbaceous at Cornale and herbaceous-shrubby at Poggio Tabor (Table S2 and S3, respectively). At all sites, soils were classified as *Technosols* (IUSS WRB Working Group (2022)) these were therefore highly incoherent, heterogeneous, and showed an evident alteration of the profile. Furthermore, a consistent fraction of gravel was observed in the top 15 cm of soil at Poggio Tabor (about 60 %), whereas at the Cornale site, a lower amount was recorded (about 10 %).

2.2. Experimental design

A randomized block design of 9 plots was established at each site, each plot measuring 3 m × 3 m. The three treatments were 1) Control (C): no treatment; 2) bioactive basalt flour (BBF) and 3) bioactive basalt flour and cover crop (BBF + CC). Each treatment was randomly replicated three times at each site. Basalt flour (Farina di Basalto® micronized by Basalti Orvieto srl) consisted in Si, Al, K, Fe, Ca, Mg, Na oxides at 45–49 %, 20–26 %, 8–10 %, 5.2–8.5 %, 7.5–8.5 %, 1.9–2.6 %, 2.2–4.9 % concentration, respectively, and showed an EC of 1.14 dS m^{-1}, pH 9.0, and CEC of 9.0 cmol kg^{-1}.

The choice of basalt flour is based on the fact that it has two fundamental characteristics that determine its remarkable effectiveness in restoring soil fertility: the first is that it has a complete and balanced content of essential trace elements for life in the soil (Fe, K, P, Ca Zn, Mg, Mn, B and others); the second is linked to the structure of the mineral which is microcrystalline or, even, glassy. This unique feature, not found in other volcanic rocks, allows the continuous release of the elements, in the form of oxides, immediately available for plant nutrition.

As reported, the term bioactive basalt flour (BBF) refers to basalt powdered rock combined with a microbial consortium; this was considered a necessary preliminary step aimed at triggering the biogeochemical processes responsible for soil biological activity. In particular, BF was added in December 2019 and December 2020 while the microbial consortium supply was performed on the following dates: December 2019, April, May, June, December 2020, May, June, September, and October 2021. The microbial consortium used is derived both from bacterial cultures from vaccine manure collected from animals not subjected to regular antibiotic and grazing therapies and from microorganisms extracted from organic soils through culture media selective for the main genera useful for the proper development of the soil microbiome. The microbial consortium was specifically formulated at Basalti Orvieto s.r.l., its maturation was obtained by defining optimal redox conditions (pH, temperature, humidity, O_2/CO_2) and nutrient

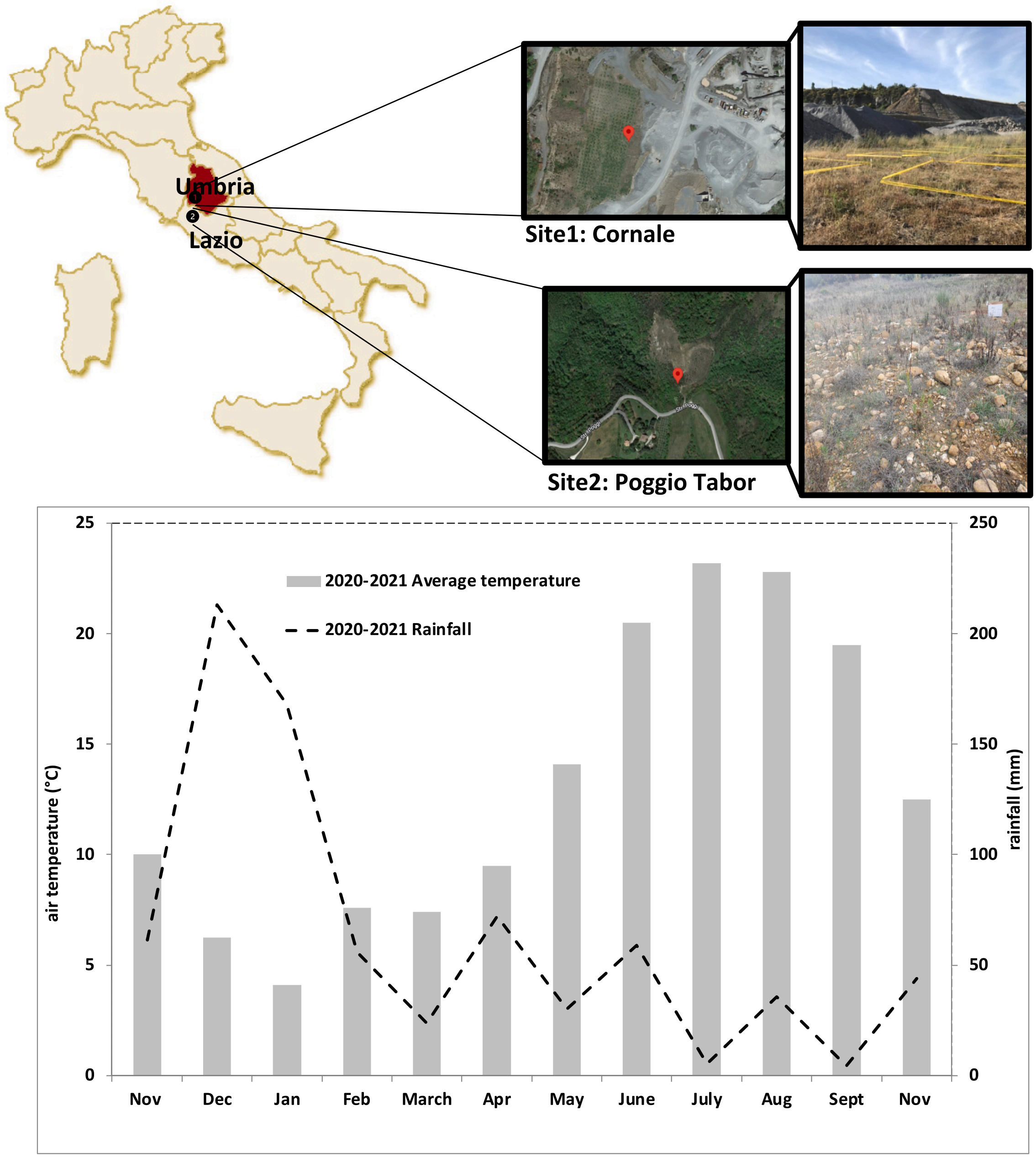

Fig. 1. Geographical and climatic data of the study area. Top left side: position of the study area within Italy. Top right side: Google Earth Maps and photographs of the two sites (Cornale and Poggio Tabor). Down: average temperature and rainfall of the area relative to the 2020–2021 years.

content. It was fermented and concentrated and consisted in lactic acidobacteria (*Lactobacillus* spp., 1×10^6 CFU g^{-1}), yeasts (*Saccharomyces* spp., 1×10^6 UFC/g) and a consortium of composting microbes (total microbial count: 1×10^9 CFU g^{-1}). The inoculum was supplied at 6 ml m^{-2} (60 l ha^{-1}) dose while basalt flour at 0.3 kg m^{-2} (3 t ha^{-1}), so each plot received BBF as 54 ml of microbial consortium + 2.7 kg of basalt flour at each supply date as described above.

During the experiment the following management practices were performed: the area has been tilled with a superficial harrowing at the start of the experiment, BBF has been applied on the surface of the soil. Before the CC was sowed, all plots had been tilled on the surface (first 15—20 cm of soil). The CC was mowed at the end of flowering and left on the soil surface.

The composition of the CC chosen for the third treatment is reported in the Supplementary materials (Table S1). During the length of the experiment, the cover crop rooting and growth was fair at Cornale while it scarcely succeeded at Poggio Tabor, probably due to the geomorphology of the site combined with a very dry Summer.

Meteorological data were collected in a weather control unit located halfway between the two sites during the experimental period and are reported in Fig. 2.

2.3. Soil sampling

The experimental design was set up in December 2019. Soil samples were collected in the Summer and Winter of 2021. Data presented in this article are related to the second year of the project. Chemical analyses were performed at the first sampling date, while biochemical ones were repeated at the two dates to account for seasonal variations due to the different management.

Soil samples were collected at four points within about 10 m^2 plot. At each collecting point, the surface soils were collected at a maximum of

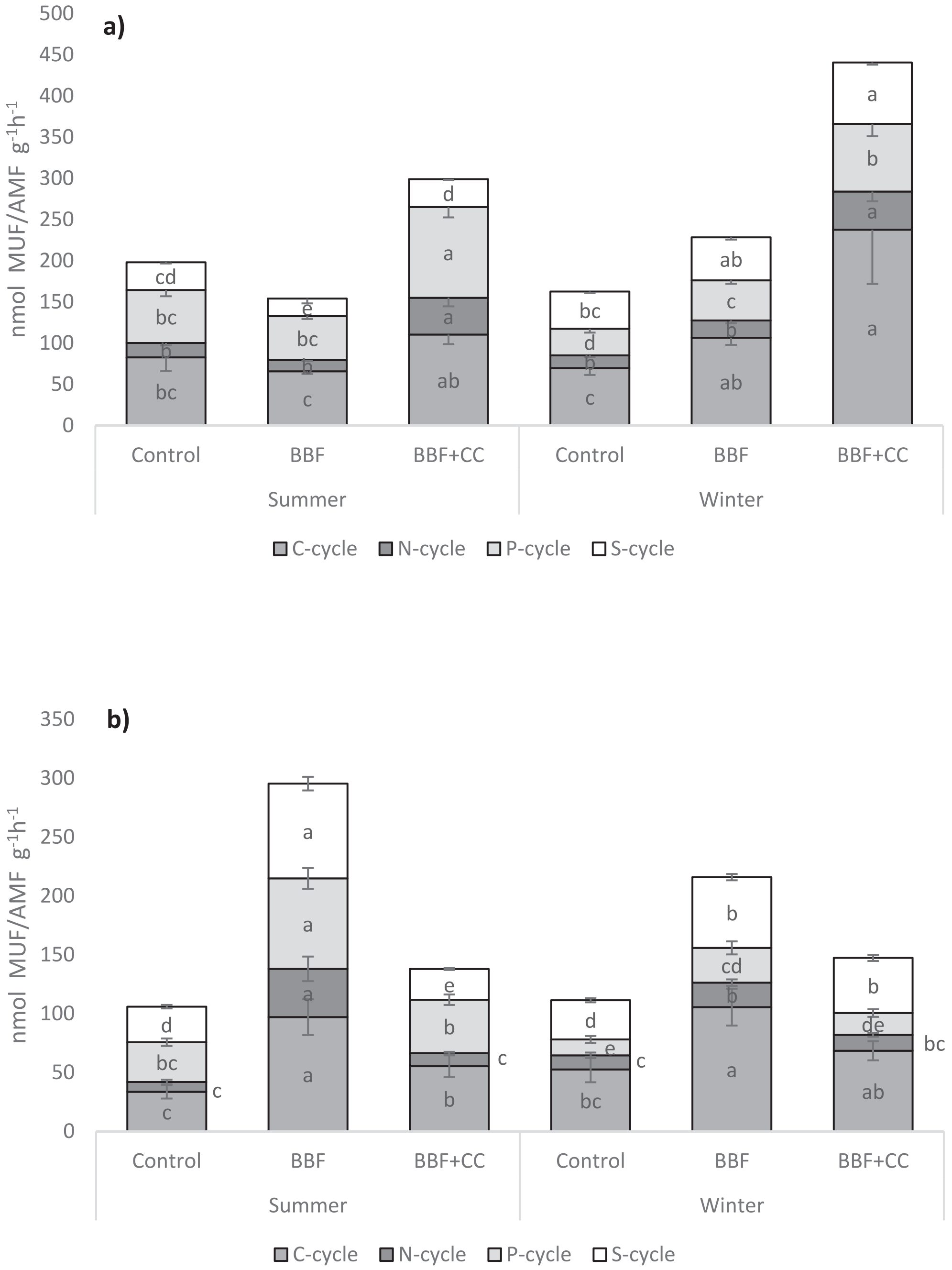

Fig. 2. Soil enzymatic activities grouped for C, N, P, and S cycling measured at different sites (a) Cornale, b) Poggio Tabor), treatments (BBF and BBF + CC), and seasons (Summer, Winter). For each enzymatic group, columns offset with different letters significantly differ for $p < 0.05$.

15 cm depth. The soil from the 4 pits of the same plot was then pooled in a mixed sample of about 500 g. Therefore, a total of 27 soil samples were brought to the laboratory at each sampling date.

Soil samples were air dried and sieved at 2 mm mesh. Prior to biochemical analyses soil samples were remoistened at 60 % of their water holding capacity and incubated at room temperature for five days.

2.4. *Chemical and biochemical indicators of soil quality*

Physical and chemical analyses of soils included: texture, pH(H_2O), organic C (TOC), total N content (TN), C and N extractable (C_{ext} and N_{ext} respectively), electrical conductivity (EC), cation exchange capacity (CEC), available P (P_{av}), and exchangeable elements as Ca_{ex}, Mg_{ex}, Na_{ex}, K_{ex}. Texture analysis was carried out in accordance with the Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2014). Soil pH was measured potentiometrically in a 1:2.5 (w/v) soil-deionised water suspension (van Reeuwijk, 2002). Total organic C (TOC) and nitrogen content (TN) were determined by means of an elemental analyser vario-MACRO cube Elementar. Available phosphorus was assessed after acid extraction with 1 M NH_4F (Bray and Kurtz, 1945). Cation exchange capacity (CEC) was measured after extraction with $BaCl_2$ pH 8.1. The electrical conductivity (EC) was determined by a WTW multi 340i conductivity meter (Weilheim, Germany) in a 1:2.5 soil:water suspensions (w:v). Exchangeable elements content was assessed by means of ICP-OES. Briefly, 5 g of each soil sample was extracted by 25 ml 1 M ammonium acetate (CH_3COONH_4) (pH 7.0) (van Reeuwijk, 2002). Each sample was orbitally shaken for 30 min at 150 rpm and then filtered through Whatman no. 42 filter paper. Ca, Mg, Na, and K exchangeable bases were determined by means of ICP-OES (8000 DV, PerkinElmer, Shelton, CT, USA)) equipped with a Scott nebulizer.

Soil microbial biomass carbon (MBC) was assessed following Vance et al (1987) by means of fumigation extraction method. On soil K_2SO_4 0.5 M extracts, deriving from not fumigated soils, extractable C and N forms (C_{ext} and N_{ext}) were assessed and used as proxies of C and N labile pools. The microbial quotient (*q*MIC, MBC-to-organic C ratio) was also measured. Microbial basal respiration (R_{mic}) was measured by incubating 10 g of soil at controlled temperature and humidity for 25 days (Badalucco et al., 1992). The CO_2 evolved was trapped, after 1, 3, 7, 10, 15 days of incubation, in 2 ml 1 M NaOH and determined by titration of the excess NaOH with 0.1 M HCl. The total CO_2 evolved at the end of the experiment is considered the cumulative respiration (MR_{cum}) while the average hourly CO_2 output is the basal respiration (BR). The enzymatic activities were measured using the fluorogenic methylumbelliferyl (MUF)-substrates method (Marx et al., 2001). The following hydrolytic enzymes were analysed: β-cellobiohydrolase (CELL; EC 3.2.1.91), N-acetyl-β- glucosaminidase (NAG; EC 3.2.1.30), β-glucosidase (β-GLUC; EC 3.2.1.21), α-glucosidase (α-GLUC; EC 3.2.1.20), β-xylosidase (XYL; EC 3.2.2.27), acid phosphatase (AP; EC 3.1.3.2), butyrate estherase (BUT; EC 3.1.1.1) and leucine aminopeptidase (LAP; EC 3.4.11.1). The respective substrates were 4-MUF β-D-cellobioside, 4-MUF-N-acetyl-

Table 1
Main chemical characteristics of the soils at different treatments (BBF and BBF + CC) and at Cornale and Poggio Tabor sites. Numbers in parentheses are the standard errors (n = 3). For each line, mean values with different letters significantly differ for P < 0.05.

				Cornale			Poggio Tabor		
		Pr(>F)		Control	BBF	BBF + CC	Control	BBF	BBF + CC
Texture				loam	sandy clay loam	sandy loam	sandy clay loam	sandy clay loam	sandy clay loam
TOC (g kg^{-1})	Treatment	0.895		3.70(0.05)b	3.99(0.05)b	4.39(0.06)b	5.53(0.09)ab	7.53(0.29)a	4.53(0.12)ab
	Site	0.037	*						
	Treatment*Site	0.444							
TN (g kg^{-1})	Treatment	0.901		0.53(0.01)ab	0.46(0.01)b	0.49(0.01)b	0.67(0.02)ab	0.95(0.04)a	0.58(0.01)ab
	Site	0.069							
	Treatment*Site	0.535							
C_{ext} (μg C g^{-1})	Treatment	<0.001	***	122.3(4.9)bc	140.9(6.7)b	174.9(12.7)a	102.7(3.3)c	124.2(12.2)bc	115.6(7.6)bc
	Site	<0.001	***						
	Treatment*Site	0.06							
N_{ext} (μg C g^{-1})	Treatment	0.197		10.3(1.5)ac	13.0(2.9)ab	18.3(3.5)a	6.3(1.7)c	8.6(1.4)ac	8.5(2.5)bc
	Site	0.006	**						
	Treatment*Site	0.447							
pH(H_2O)	Treatment	0.009	**	6.3(0.02)c	6.4(0.04)b	6.45(0.04)b	7.1(0.03)a	7.2(0.03)a	7.1(0.01)a
	Site	<0.001	***						
	Treatment*Site	0.098	*						
CEC (cmol(+) kg^{-1})	Treatment	0.002	**	7.6(1.1)bc	7.8(0.9)bc	4.2(1.5)d	6.3(2.2)cd	13.4(0.3)a	10.6(1.0)ab
	Site	0.003	**						
	Treatment*Site	<0.001	***						
EC (μS cm^{-1})	Treatment	<0.001	***	380.6(12.2)c	413.3(15.0)c	523.2(17.6)b	1029(28.4)a	998(1.2)a	1064(38.3)a
	Site	<0.001	***						
	Treatment*Site	0.038	*						
P_{av} (μg g^{-1})	Treatment	0.650		5.6(0.4)a	6.3(0.3)a	6.5(0.4)a	2.6(0.7)b	2.0(0.4)b	2.2(0.2)b
	Site	<0.001	***						
	Treatment*Site	0.651							
Mg_{ex} (μg g^{-1})	Treatment	0.723		192.8(11.6)b	232.1(19.9)a	207.3(8.1)ab	100.0(9.8)c	83.5(3.5)c	95.6(2.8)c
	Site	<0.001	***						
	Treatment*Site	0.02607	*						
K_{ex} (μg g^{-1})	Treatment	0.3062		383.4(20.8)a	555.4(87.8)a	434.8(21.9)a	115.2(17.6)b	113.0(7.3)b	134.0(21.4)b
	Site	<0.001	***						
	Treatment*Site	0.3062							
Ca_{ex} (μg g^{-1})	Treatment	0.31329		1340(77.0)c	1512(147.8)c	1473(69.2)c	4537(218.9)a	4581(58.1)a	4092(178.4)b
	Site	<0.001	***						
	Treatment*Site	0.08432							
Na_{ex} (μg g^{-1})	Treatment	0.04783	*	225.2(11.9)a	272.1(21.8)a	223.0(17.4)a	69.1(13.5)b	43.0(3.3)bc	34.2(1.8)c
	Site	<0.001	***						
	Treatment*Site	0.01125	*						

TOC = total organic carbon, TN = total nitrogen, C_{ext} = C extractable in K_2SO_4, N_{ext} = N extractable in K_2SO_4, CEC = cation exchange capacity, EC = electrical conductivity, P_{av} = available Phosphorous, Mg_{ex} = Mg exchangeable, k_{ex} = K exchangeable, Ca_{ex} = Ca exchangeable, Na_{ex} = Na exchangeable.

β-glucosaminide, 4-MUF β- D-glucoside, 4-MUF α-D-glucoside, 4-MUF-7-β-D-xyloside, 4-MUF-phosphate, 4-MUF-butyrate and L-leucine-7-amino-4-methylcoumarin (AMC). Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Fluoroskan Ascent, Labsystem, Frankfurt, Germany) after 0, 30, 60, 120 and 180 min (Marinari et al., 2013). The results were expressed as nmoles of product (MUF or AMC) of each enzymatic reaction released per g of soil per unit of time in relation to a standard curve performed with increasing MUF or AMC concentrations and incubated at the same experimental conditions. The ecoenzymatic C/N and N/P acquisition activities were measured by the ratios of β-glucosidase/(chitinase + leucine aminopeptidase) [β-gluc/(NAG + LAP)] and (chitinase + leucine aminopeptidase)/acid phosphatase activities [(NAG + LAP)/Phosph], respectively (Sinsabaugh et al., 2009). These ratios represent the microbial limitation for N with respect to C and P, respectively.

2.5. Statistical analysis

To test the differences among chemical and biochemical soil properties a two–way analysis of variance (ANOVA) was performed. For the chemical properties, we considered the site and treatment variables, for biochemical analysis season and treatment variables. The normality and the homoscedasticity of the data were verified by graphical analysis of residuals. When these assumptions were not satisfied, the logarithmic transformation was selected by the maximum likelihood procedure devised by Box and Cox (1964), as implemented in the Box-Cox function of the package Modern Applied Statistics with S (MASS) (Venables and Ripley 2002) in the R statistical environment (R Core Team, 2020). All significant effects were assessed by Fisher's post-hoc test at $p < 0.05$~~.~~ The statistical analyses were performed using the R 3.5.0 statistical software (R Core Team, 2020).

3. Results

3.1. Chemical indicators

The main chemical characteristics of Cornale and Poggio Tabor sites are shown in Table 1. The TOC and TN values were not statistically different between treatments, but in Poggio Tabor BBF treated plots the TOC and TN contents were significantly higher than in Cornale treated plots. As for the extractable forms BBF + CC at Cornale showed the highest C_{ext} and N_{ext} values (174.9 μg C/g and 18.3 μg C/g respectively).

The pH was higher at Poggio Tabor with respect to Cornale, and, within Cornale, the two treated plots (BBF, BBF + CC) showed a slightly significantly higher value than the control plot. The CEC showed two different behaviours in the two sites. At Cornale the BBF + CC plot showed lower values (4.2 $cmol_{(+)}$ kg^{-1}), conversely, at Poggio Tabor, the two treated plots showed higher values (BBF = 13.4 and BBF + CC = 10.6 $cmol_{(+)}$ kg^{-1}) than the control (6.3 $cmol_{(+)}$ kg^{-1}) (Table 1). The EC was always higher at Poggio Tabor with respect to the Cornale site without differences among treatments, whereas the BBF + CC plot showed the highest value at Cornale (1064 μS cm^{-1}). The available P, Mg_{ex}, K_{ex}, Na_{ex} showed higher values at Cornale with respect to Poggio Tabor, conversely, Ca_{ex} was lower at Cornale with respect to Poggio Tabor, without differences between treatments (Table 1).

3.2. Biochemical indicators

At Cornale the enzymatic activity was higher in BBF + CC plots with respect to the other treatment and control in both seasons (Fig. 2a). The higher activity in BBF + CC plots was more evident in Winter and was mainly due to the enzymes linked to C cycle (β-glucosidase, α-glucosidase, β-xylosidase, and β-cellobiohydrolase) (237.6 nmol MUF $g^{-1}h^{-1}$), whereas in Summer the differences with control plots were mainly attributable to the enzymes linked to N cycle (N-acetyl-β- glucosaminidase, leucine aminopeptidase) (44.6 nmol MUF/AMF $g^{-1}h^{-1}$) and P (110.1 nmol MUF $g^{-1}h^{-1}$) (acid phosphatase) (Fig. 2a). At Poggio Tabor the plots that recorded a greater activity were those treated with basalt flour and the microbial inoculum in both seasons, even if in Winter no significant differences were observed between the two treatments (Fig. 2b). Butyrate esterase activity showed the highest values in the BBF + CC plots in both seasons at Cornale (1150.5 nmol MUF $g^{-1}h^{-1}$ in summer and 1162.3 nmol MUF $g^{-1}h^{-1}$ in Winter), and in the two treated plots in Summer at Poggio Tabor (Fig. 3a). As regards the ratio of β-gluc/(NAG + LAP) there were no significant differences at Cornale among treatments, while at Poggio Tabor, in Winter, the values were higher than in Summer (Fig. 3b). Fig. 3c showed that the (NAG + LAP)/Phosph ratio had the same trend in the two sites with lower values in Winter in the BBF plots. F ig. 5a shows microbial basal respiration (BR) with an increasing trend starting from control to the BBF + CC plots in both seasons at Cornale, while at Poggio Tabor it reached the highest values in both treated plots in Summer (0.35 μg C-CO2 $g^{-1}h^{-1}$ in BBF plots, and 0.39 μg C-CO2 $g^{-1}h^{-1}$ in BBF + CC plots). As for the two selected microbial indexes, the microbial quotient ($q$mic) showed significantly higher values at Cornale in summer with respect to the Winter with an increase in BBF + CC plots (+8.8 %) while at Poggio Tabor, although, with lower values, a significant effect of both treatments was evident always in Summer (F ig. 5b). Finally, the metabolic quotient (qCO_2) showed the highest value in BBF + CC plots in Winter at Cornale while it showed no significant differences among treatments and seasons at Poggio Tabor (Fig. 4c).

4. Discussion

4.1. Effect on chemical indicators

The very low content of soil total organic carbon and total nitrogen found in both sites was also reported at a mining site by Sheoran et al., (2010). The soils of the quarry sites are subject to strong anthropogenic pressure and are characterised by low organic matter and nutrient content as also reported by Peco et al., (2021).

Total organic carbon and total nitrogen are chemical indicators of soil quality that show significant changes to treatments over rather long periods of time; they are in fact defined as static, or moderately dynamic, indicators (Bünemann et al., 2018). The short period of this experiment therefore did not allow significant changes in their content to be appreciated. Conversely, to appreciate treatment-induced changes of the organic C and N pools, the determination of extractable C and N fractions, which represent labile C and N pools, has been performed. Only at Cornale an increase in C_{ext} was observed due to the CC growth and green manuring. This increase could be mainly attributed to exudation of labile C compounds such as carbohydrates, aminoacids, aliphatic or aromatic organic acids, phenols, and fatty acids (Haynes, 2005) derived from plant roots. This C input from the rhizosphere represents an investment made by the plant to modify soil conditions and establish an appropriate environment for its development (Massaccesi et al., 2015). At Poggio Tabor, an effect due to the addition of the plants could not be observed, as the site-specific conditions severely impaired their development (Toktara et al., 2016).

Only at the Cornale site, where the starting pH value was sub-alkaline, it was observed an increase in soil reaction combined with an increase in some nutrients content after the BBF addition. These increases could be attributed to the chemical properties of the basalt flour, confirming a potential nutrient supply to the soil. The increase in pH value following the addition of rock flour is widely reported in the literature (Hinsinger et al.,1995; Gillman et al., 2002; Panhwar et al., 2014; Maliszewski, 2021; Wotchoko et al., 2021; Swoboda et al., 2022; Gindri Ramos et al., 2022). However, the most significant and remedial effects, in situations of severely impaired fertility, are highlighted in acidic soils, Oxisols (Shamshuddin and Anda, 2012; Anda et al., 2015).

An increase in CEC was only visible at Poggio Tabor treated plots, probably due to the pH values that ranged from neutral to sub-alkaline

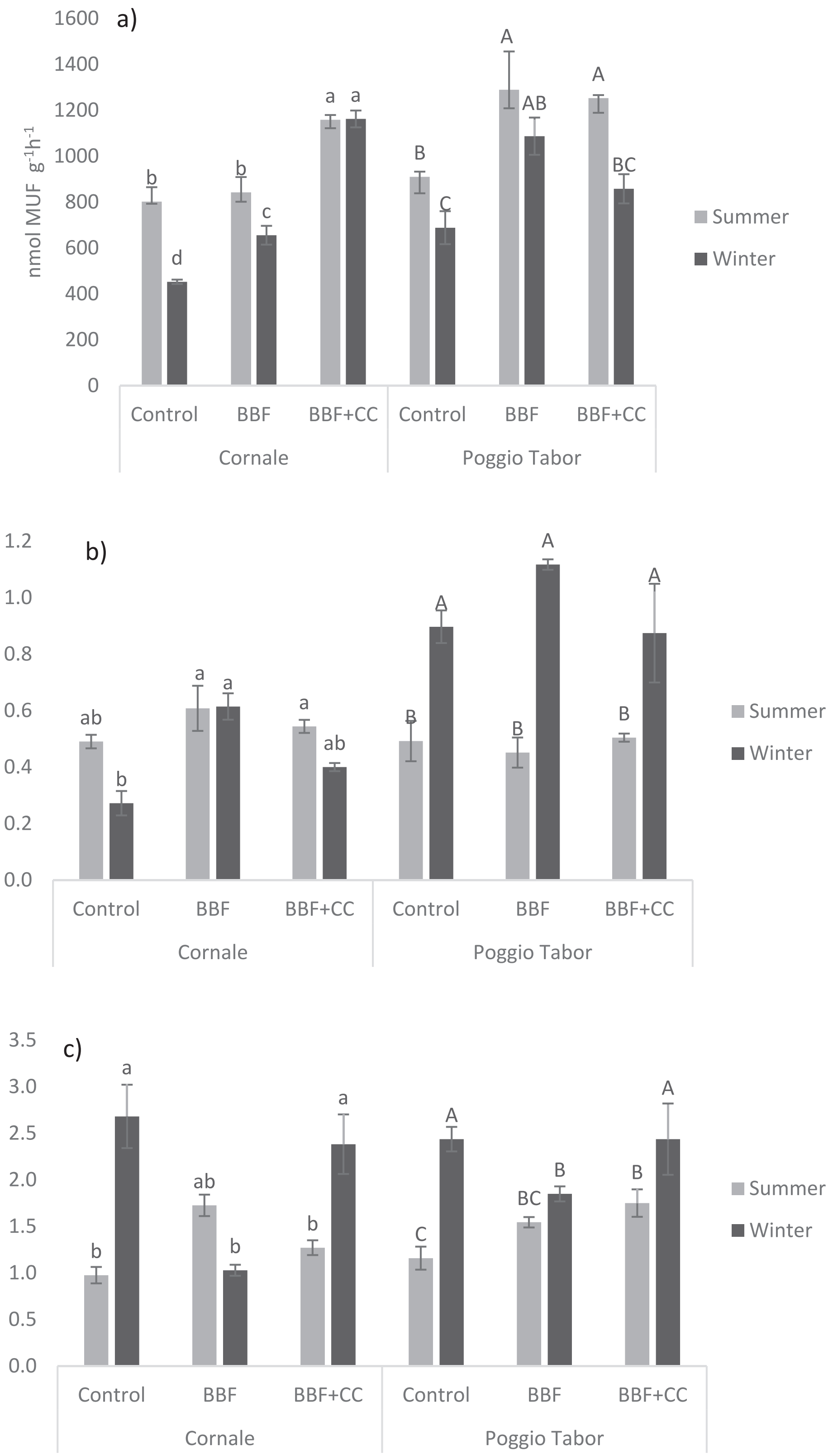

Fig. 3. A) soil butyrate esterase activity, b) stoichiometric ratios ß-gluc/nag + LAP, and c) NAG + LAP/Phosph measured at different sites (Cornale, Poggio Tabor), treatments (BBF and BBF + CC) and seasons (Summer, Winter). Error bars are the standard errors ($n = 3$). Within the Cornale site, different lowercase letters mean significant differences at $p < 0.05$. Within the Poggio Tabor site, different capital letters mean significant differences at $p < 0.05$.

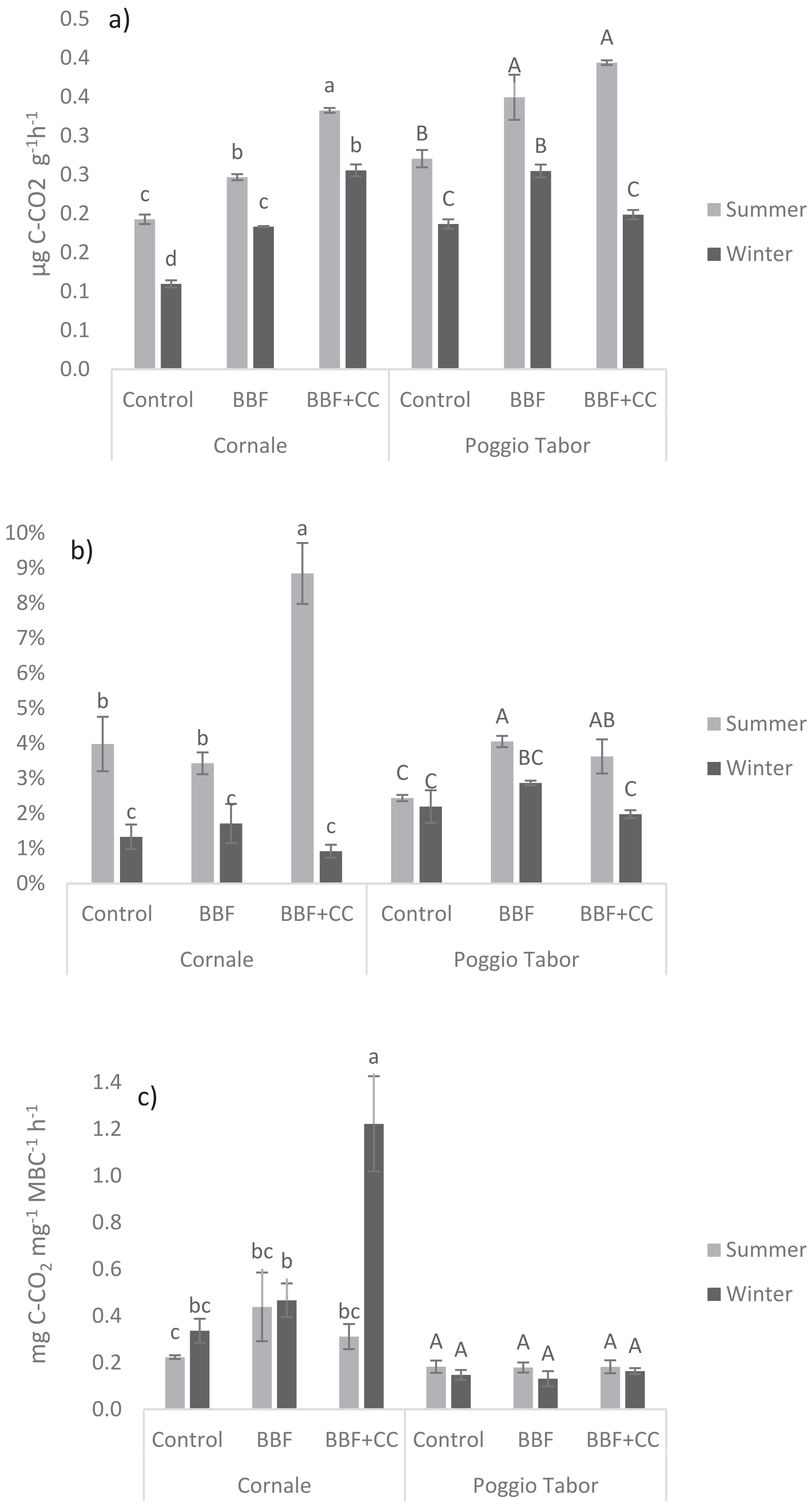

Fig. 4. A) soil microbial basal respiration (BR), b) microbial quotient (q_{mic}), and c) metabolic quotient ($q\mathrm{CO}_2$) at different sites (Cornale, Poggio Tabor), treatments (BBF and BBF + CC) and seasons (Summer, Winter). Error bars are the standard errors ($n = 3$). Within the Cornale site, different lowercase letters mean significant differences at $p < 0.05$. Within the Poggio Tabor site, different capital letters mean significant differences at $p < 0.05$.

level. Some studies claim that the release of exchangeable cations Ca, Mg, K, and Na from the basalt flour added to the soil can increase the availability of exchangeable elements retained by the soil (Gillman et al., 2002; Silva et al., 2012). The significant decrease of CEC in BBF + CC plots at Cornale may be due to the intense nutrient requirement of the cover crop. The electrical conductivity values were always higher at the Poggio Tabor site with respect to Cornale, probably due to a high percentage of gravel and a sandy clay loam texture that, favoring a faster water flow rate, may promote limestone weathering as also confirmed by the three-fold higher exchangeable Ca concentration.

Conversely, at Cornale, an increase in EC in BBF + CC plots was observed; this increase could be due to the uptake by plants of the available water leading to a salt concentration.

The available P content was very low at both sites with values below 6.5 mg kg^{-1} and at Poggio Tabor site with values lower than 2.6 mg kg^{-1} regardless of treatments. In this last case, the very low values of P content found may be ascribable to the more alkaline pH with respect to Cornale, which has reduced the availability of this element, making it insoluble or due to its rapid assimilation by the added microbial biomass and/or by the plants.

Ramos et al. (2022) reported that the alteration rate of the added rock dust may be influenced by additional environmental factors. Therefore, the agronomic effectiveness of the remineralization process by rock dust/flour added to soil is directly related to its mineralogy, chemical composition, soil type, and environmental conditions that accelerate, or not, the alteration processes (Gindri Ramos et al., 2022). For example, in tropical soil and climate contexts characterized by high temperatures and rainfall, together with the contribution of plants and microorganisms, these processes are accelerated. Campbell (2009), for example, does not report any positive effects on the nutrient content of the soil as a result of the addition of rock dust, nor in the nutrient content of the crops under study.

4.2. *Effect on biochemical indicators*

As a general consideration, in this study all groups of soil enzymes, averaged across both treatments and sites and related to the control, were positively activated according to the following ranking (data not shown): N > C > P > S cycling enzymes. An enhancement of hydrolytic activities related to the main biogeochemical cycles is an indicator of nutrient acquisition activity performed by soil microbes (Sinsabaugh et al., 2009).

At Cornale, an increase of enzymatic activities was observed in BBF in Winter and in BBF + CC plots in both seasons. The results are in line with another study in which rock flour, as a source of macronutrients, was used in combination with organic compounds to test its effects on the metabolic activity of microorganisms resulting in an increase in enzymatic activity (Li et al., 2021). C cycling enzymes showed the largest increase during the Winter season. This last result could be due to improved soil moisture conditions that favour enzymatic activities. Sardans and Peñuelas (2005) also report that enzymes are highly dependent indicators of rainfall conditions. The increased C-cycling enzyme activity in presence of cover crop could be due to an increased substrate availability derived from rhizodeposition stimulated by the presence of root and shoot growth. In this regard, Sinsabaugh and Shah (2011) reported that in grassland soils, the cellulose represents a significant portion of the plant litter reaching the soil, and the production of β-glucosidase is necessary because it catalyses the hydrolysis of cellobiose to glucose.

Moreover, at Cornale, in Summer, enzymatic activities involved in N, P, and S cycling, were particularly stimulated by the presence of the cover crop indicating a high demand for these nutrients either by the plant biomass or microbial pool. Under soil oligotrophic conditions plants and/or microbes release hydrolytic enzymes able to release inorganic nutrients (e.g. N, P, S) from organic compounds. This result is in accordance with Castellano Hinoiosa and Strauss (2020), reporting that the use of the cover crop can positively influence the N cycling promoting increased nitrogen availability in the soil to be used by the microorganisms and plants.

Conversely, at Poggio Tabor, the higher increase in enzymatic activity involved in the nutrients biogeochemical cycles was evident only in BBF plots, and particularly during Summer season. This result could be due, as previously mentioned, to the almost total absence of the cover crop growth in that site and suggests that in particularly hostile and arid conditions the biotic action is carried out by those living forms that can resist adverse conditions such as selected microbial groups. At this site, in Summer, BBF plots showed the highest activities of arylsulfatase and chitinase which were also significantly correlated to each other ($r = 0.88$, $P < 0.001$, data not shown). Ester sulphates (substrates of arylsulphatase) and chitin (substrate of chitinase) are present only in fungal cells and are their major constituent (Bandick and Dick, 1999). This result suggested a constraint of fungal biomass growth (*Saccharomyces* spp) added with the inoculum. The drought conditions that characterized Summer 2021 may have limited microbial inoculum growth, in particular fungal biomass which started to be decomposed.

Finally, butyrate esterase activity, which may be considered a proxy of endocellular activity (Wittmann et al., 2004), was highly active in BBF + CC at Cornale, and in BBF at Poggio Tabor, in both seasons, indicating that the addition of BBF triggered the metabolic activity of soil microbial biomass. In accordance with Kähkonen et al. (2007), this hypothesis was also confirmed by the positive significant correlation with our data on basal respiration ($r = 0.66$, $P < 0.001$, data not shown).

Soil microbes were N-limited in the soils of both quarries when compared to C and to P requirements. In fact, as both ecoenzymatic C/N and N/P acquisition activities showed, for both sites and seasons the values of these ratios were, beyond each threshold limit (C/N <1.41 and N/P >0.44, Zeglin et al., 2013; Sinsabaugh et al., 2009). Although this limitation was not eliminated by the treatments, an improvement of both ratios was evident in Winter in BBF plots, significant only at Poggio Tabor.

As regards the microbial quotient, it does not show significant seasonal differences at Poggio Tabor, while a significant increase of this quotient was found at Cornale in Summer in BBF + CC plots, corresponding to the maximum physiological activity of the vegetation cover and thus the release of root exudates.

Furthermore, in June the cover crop was mowed, thus the additional flush of C-rhizodeposits released by roots, caused by the stress induced by the removal of the epigeic portion, increased the availability of labile C forms. This led to a process of C immobilization within native and added microbial biomass enhancing *q*mic. An increase of microbial biomass C following Mediterranean grassland mowing was also observed by Gavrichkova et al. (2010).

The positive effect of the cover crop is confirmed by several studies in which its use resulted in an increase in the microbial biomass content due to the increased amount of organic matter supplied to the soil (Chavarría et al., 2016; Frasier et al., 2016).

Conversely, at Poggio Tabor, the microbial quotient showed higher values in the treated plots only in Summer. At this site, the microrganisms added with the inoculum couldn't benefit from any carbon substrates derived from the cover crop, as already reported, but were, probably, supported by the slightly higher amount of total organic carbon found in this site.

An increase in the rate of soil respiration was observed in both seasons and treatments at Cornale with a higher rate in BBF + CC. Maliszewski (2021) reported that basalt flour positively influenced the respiration rate of the soil and that this effect was related to the concentration of the flour used since an increase in its quantity corresponded to an increase in O_2 consumption by microorganisms. The results obtained agree with other studies in which the use of cover crops determined an increase in CO_2 fluxes due to the decomposition processes of organic matter by microorganisms (Steenwerth and Belina, 2008). In fact, as reported by Alvarenga et al. (2014) in degraded soils there is a direct relationship between the addition of organic matter and the stimulation of microbial community growth and activity, resulting in an increase in mineralisation activity.

In this study, a large significant increase in metabolic quotient was observed only at Cornale in the BBF + CC plots. The release of easily available C compounds from plant roots acted as a priming effect triggering microbial decomposition activity thus raising this quotient. Previous research has shown that the addition of fresh organic inputs can accelerate the decomposition of stable SOM (Kuzyakov, 2010; Zornoza et al., 2017). The use of soil quality bioindicators allowed us to highlight the likely mechanisms occurring in the soil environment after the treatment with BBF + CC may involve mineral nutrient release from basalt flour followed by plant root uptake. This triggers plant development and an increase of root exudation activity that fuels microbial biomass in terms of labile C compounds availability. Native and added microorganisms benefit from these organic C sources and mineral nutrients from basalt flour increasing their biomass and metabolic activity in terms either of respiration or enzymatic activities.

5. Conclusions

The joint application of BBF and wild plants or cover crops could be the initial step toward establishing a natural system for the restoration of degraded soils. As hypothesized, bioindicators of soil quality proved more effective than chemical indicators in promptly responding to treatments. These fluctuations were influenced by seasonal variations, pedoclimatic conditions, and the diverse development of cover crops at both experimental sites. Consequently, the use of early predictors for changes in soil biogeochemical processes demonstrated that BBF improved soil quality in the short term by enhancing nutrient cycling and promoting microbial growth and activity. The effectiveness of this reclamation strategy is highly reliant on site-specific conditions, including lithological substrate, climate, pH, and the organic matter content native to the area. A primary limitation of this study is the confined area in which basalt flour and microbial inoculum were tested. Future research should focus on implementing BBF on a larger scale for restoration purposes. Furthermore, to enhance soil quality and nutrient cycling in severely degraded soils such as abandoned quarries, it is imperative to provide an external organic carbon source and utilize a microbial consortium to facilitate the restoration of the vital ecosystem processes. In conclusion, the use of basalt flour combined with a microbial inoculum proves to be a promising strategy for revitalizing degraded soils; this practice is further advantageous in terms of waste recovery as the flour is derived from the micronization of basalt processing wastes. A future research activity could move in the direction of testing BBF on different soil types, climatic conditions, and especially, in agricultural soils to assess restoration of fertility for production purposes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2024.107820.

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