

1 **Effects of pumice mining on soil quality**

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3 **A. Cruz-Ruíz¹, E. Cruz-Ruíz¹, R. Vaca², P. Del Aguila², and J. Lugo²**

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5 ¹Instituto de Ingeniería, Universidad Autónoma de Baja California. Mexicali, Baja
6 California, México

7 ²Facultad de Ciencias, Universidad Autónoma del Estado de México, Instituto
8 Literario 100 Toluca, 50 000 México

9
10 *Correspondence to:* J. Lugo (jorgelug@gmail.com)

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13 **Abstract.** Mexico is the world's fourth most important maize producer; hence,
14 there is a need to maintain soil quality for a sustainable production in the upcoming
15 years. Pumice mining is a superficial operation that modifies large areas in Central
16 Mexico. The main aim was to assess the present state of agricultural soils differing
17 in elapsed-time since pumice mining (0–15 years), in a representative area of the
18 Calimaya region in the State of Mexico. The study sites in 0, 1, 4, 10 and 15 year-
19 old reclaimed soils were compared with adjacent undisturbed site. Our results
20 indicate that soil organic carbon, total nitrogen, microbial biomass carbon and
21 microbial quotients were greatly impacted by disturbance. A general trend of
22 recovery towards the undisturbed condition with reclamation age was found after
23 disturbance, being the recovery of soil total N faster than soil organic C. Principal
24 components analysis was applied. The first three components gathered explain
25 71.72 % of the total variability. The first factor reveals strong associations between
26 total nitrogen, microbial biomass carbon and pH. The second factor reveals high
27 loading of urease and catalase. The obtained results revealed that the most
28 appropriate indicators to diagnose the quality of the soils were: total nitrogen,
29 microbial biomass carbon and soil organic carbon.

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1 1 Introduction

2
3 Land degradation refers to a process induced by human activities that cause the
4 decrease of biological productivity or biodiversity, as well as the current capacity
5 and/or potential to sustain human life (Oldeman, 1998). Land degradation and
6 desertification affect many regions of the world (Cerdà et al., 1999; Bai et al., 2013;
7 Izzo et al., 2013; Wang et al., 2013; Yan et al., 2015) and there is a need to restore
8 those areas affected by land degradation processes (Guénon et al., 2013; Özcan
9 et al., 2013; Kröpfl et al., 2013; Li et al., 2013; Tejada et al., 2014; Zucca et al.,
10 2015). There are examples of studies in opencast coal mines, magnesite mines
11 and limestone mines (Haigh et al., 2013; Martin-Moreno et al., 2013; Milder et al.,
12 2013; Raizada and Juyal, 2013; Mukhopady and Maiti, 2014; Pallavacini et al.,
13 2014; Wick et al., 2014) whilst there are few studies over opencast pumicite
14 mines.

15 The open pit mining has great economic importance in Mexico therefore taking
16 the volume of production of non-metallic minerals as an indicator, the State of
17 Mexico is the largest producer of non-metallic minerals mainly extracted i.e. sand
18 and gravel (Jimenez et al., 2006). In the agricultural area of the Municipality of
19 Calimaya highlands in the State of Mexico, opencast mining is carried out. After
20 extractive operations in the pumice areas, agricultural use is continued soil
21 although productivity declines while the landscape and soil characteristics are
22 substantially altered. These facts evidence the need of suitable correction
23 management actions to accelerate the succession process and return of the
24 degraded area to an environmentally acceptable and productive condition
25 (Rogowski and Weinrich, 1987). Pumice is a volcanic rock with trapped gas
26 bubbles formed during volcanic eruptions (Whitham and Sparks, 1986); is made up
27 of Si, Al, K, Na, Fe oxides, with a small percentage of Ca, Mg, Mn, Ti oxides
28 (Liguori et al., 1984).

29 The main causes of land degradation during mining operations are 1) removal of
30 vegetation cover and topsoil, 2) excavation and dumping of overburden, 3)
31 changes in the landscape (Mukhopadhyay et al., 2013), 4) disruption of surface

1 and subsurface hydrologic regimes, 5) transformation of fertile cultivated land into
2 wasteland and in some cases 6) serious environmental pollution and ecological
3 degradation, which can lead to the loss of biodiversity (Keskin and Makineci,
4 2009). Soil in mined sites is replaced by overburden, which differs substantially
5 from developed soils (Huggett, 1998; Keskin and Makineci, 2009), with adverse
6 properties such as severe depletion of organic matter, erosion risk, toxicity, and
7 nutrient deficiency, which commonly reduce productivity in post mining landscapes
8 (Sourkova et al., 2005).

9 In a post-mining landscape, it is necessary, the regeneration of the uppermost
10 soil layer, organ-mineral horizon, and soil biota, which transforms organic matter
11 (Frouz et al, 2001). The accumulation of organic matter (OM) is critical because it
12 results in positive changes in physical and chemical soil properties, such as water
13 holding and sorption capacities, nutrient content and availability, soil bulk density,
14 buffering capacity, increases microbial biomass and extractable carbon, microbial
15 community structure and biodiversity. Moreover, OM is an energy source for the
16 soil microorganisms, which drives decomposition and mineralization of plant
17 residues, thereby releasing nutrients (Sourkova et al., 2005; Laudicina et al., 2015)

18 Soil quality included physical, chemical and biological properties, as well as soil
19 processes and their interactions. The selection of some properties to assess soil
20 quality is an effective way. Some authors have used independent indicators, while
21 others preferred their combinations into models or expressions in which various
22 properties are involved, these expressions are called indices (Zornoza et al.,
23 2015). The establishment of multiparametric indices has been used as an
24 adequate tool for integrating greater information of soil quality. It provides a more
25 holistic measurement of soil quality (Brevik et al., 2015; Zhang et al., 2015).
26 Several studies have generated indices from a data set using physical, chemical
27 and biological indicators. Organic carbon, microbial biomass and enzymes activity
28 have been widely used to assess impact of change in land use and reclaimed soils
29 (Chodak and Niklinska, 2010).

30 Enzyme activities measurement is widely used to examine nutrient cycling
31 processes in soil (Nannipieri et al., 1990; Tabatabai and Dick, 2002). Moreover,

1 enzyme activities can provide indications of quantitative changes in SOM and are
2 usually related to the presence of viable microorganisms and their oxidative
3 activities (Gianfreda et al., 2005), which could be sensitive indicators of the effect
4 of land degradation on soil microbial activity. Soil hydrolases measurements
5 provide an early indication of changes in soil fertility, since they are related to the
6 mineralization of important elements such as nitrogen, phosphorus and carbon and
7 may provide some insight into the metabolic capacity of the soil (Shaw and Burns,
8 2006; García-Orenes et al., 2010). Urease plays an important role in soil nitrogen
9 cycle because it can hydrolyze urea an important fertilizer in agricultural systems to
10 ammoniacal nitrogen (Sinsabaugh et al., 2000, Caldwell, 2005). Catalase has a
11 great effect on changing soil redox, chemical properties of soil solution, and
12 accelerating transformation of organic matter (Wang, 2012). The metabolic
13 quotient estimates the activity and efficiency of decomposition (or C use) by soil
14 microbes (Anderson and Domsch, 1990) and has resulted a suitable indicator to
15 provide evidence of soil perturbation (Zornoza et al., 2015).

16 Little information exists about how mining affects soil in cropland regions of the
17 world and especially those of the central highlands of Mexico. The present work
18 aimed to assess the changes produced in the agricultural soils differing in elapsed-
19 time since pumice mining (0–15 years). With the information from this study we
20 examine valuable indicators of surface mine reclamation progress in open cast
21 mine.

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23

24 **2 Materials and methods**

25 **2.1 Study site**

26 This study was conducted in Calimaya, State of Mexico (Central Mexico; 19°13'25"
27 N; 99°44'02" W), where the mean annual temperature is 14 °C and the annual
28 rainfall is 800 mm (INEGI, 2009). The dominant climate is subhumid with summer
29 rains. Dominant soils are Andosols (WRB, 2014). The main type of land use in the
30 region is cropland based on maize. Cultivation techniques consist of monoculture,
31 crop residues removal, and the use of N fertilizer, herbicides and pesticides. The

1 change of use of agricultural land to urban has caused a decline in cultivated land
2 from 7 508 to 5 350 ha between 2010 and 2011 (GEM, 2011- 2012). Following
3 standard practice on surface mining sites, the topsoil was stripped and stockpiled
4 until mining operations were completed; stored soil was then spread on top of
5 overburden.

6

7 **2.2 Sampling and processing**

8

9 On February 2011, five mine sites, differing in elapsed-time since reclamation (0–
10 15 years) of opencast pumice mine spoils in the Central of Mexico, were chosen on
11 the basis of similarity of aspect as well as proximity to one another and an adjacent
12 undisturbed site, for comparison, located about 2 800–2 950 m above sea level.
13 Treatments were considered S₀, S₁, S₄, S₁₀ and S₁₅ land where the pumice
14 extracted two months, 1, 4, 10 and 15 years respectively and land no mined (S).
15 Pumice extraction is despite its depth. In all areas immediately after the removal
16 maize crop was continued under seasonal conditions. The slopes of the sampling
17 sites ranged from 25-30%. Pumice layer was located 30 to 180 cm deep.

18 Surface mining is one of the most complete forms of human-caused habitat
19 alteration and degradation. In this case, mining eliminates vegetation, removes
20 topsoil (30 cm) and overburden by excavation change topography and geological
21 structures permanently. The reclamation process involved the return of topsoil after
22 mining exploitation. These new altered soils are called reclaimed mine soils, which
23 they have a different years of agriculture use.

24 These sites had been continuously cultivated since reclamation of mine spoils,
25 surface soil samples (0–15 cm depth) were taken in February (during the dry
26 season), June (onset of the rainy season), and March 2012 (during the dry
27 season), stored at 4 °C for biochemical analyses. Afterwards, they were passed
28 through a 2 mm mesh sieve.

29 The experiment had a completely randomized design with three spatially
30 separated landscape-level plots (more of 3 ha) as replicates for each land use (n=3
31 for each land sue).

1

2 Sampling sites were selected considering the extraction pumice times; the
3 distance between them was 450 m and the area of the 3 ha. The soil sampled from
4 each field was pooled separately. Were obtained by systematic sampling at each
5 site a composite sample from 30 subsamples was collected of six treatments (S,
6 S₀, S₁, S₄, S₁₀ and S₁₅).

7 The following parameters were analyzed: gravimetric moisture content (GMC)
8 which was measured gravimetrically, water holding capacity (WHC) according to
9 Forster (1995), and soil bulk density as described by Domínguez and Aguilera
10 (1987). The pH soil and electrical conductivity were determined in soil/water (1:2.5
11 w/v), (Thomas, 1996). Content of soil organic carbon (SOC) was determined by the
12 Walkley-Black method (Nelson and Sommers, 1996) and TN with Kjeldahl
13 digestion (Bremner, 1996).

14 MBC of soil samples was estimated by chloroform fumigation and extraction
15 method (Vance et al., 1987). Basal respiration (BR) was estimated by quantifying
16 the carbon dioxide (CO₂) released by microbial respiration in 33 days of incubation
17 at 25 °C adjusted to 40 % water holding capacity (WHC). For this purpose, 25 g
18 soil was filled into flasks, together with small flasks containing 10 mL of 0.2 mol L⁻¹
19 NaOH, to capture the released CO₂, and hermetically sealed. CO₂ was determined
20 by titration with 0.2 mol L⁻¹ HCl, after precipitation of the barium carbonate resulting
21 from the addition of BaCl₂ to the NaOH solution, using phenolphthalein diluted in
22 100 mL ethanol (60 %, v/v) as indicator (Alef and Nannipieri, 1995).

23 Catalase activity was measured by titrating the residual H₂O₂ added to the soil
24 and not degraded by catalase with KMnO₄ (Johnson and Temple, 1964). Acid
25 phosphatase activity was measured by spectrophotometry (400 nm) of *p*-
26 nitrophenol released from 1.0 g soil after a 60 min incubation at 37 °C with a 0.025
27 mol L⁻¹ *p*-nitrophenyl phosphate substrate, in 4 mL of 0.17 mol L⁻¹ MUB (universal
28 buffer), at pH 5 (Tabatabai and Bremner, 1969). Urease activity was determined
29 as the amount of NH₄⁺ released from 5.0 g soil after a 120 min incubation with a
30 substrate of 0.2 mol L⁻¹ urea at 37 °C, 4.5 mL of THAM (Tris buffer) (Alef and

1 Nannipieri, 1995).The metabolic quotient (qCO_2) was calculated as the ratio of
2 basal respiration to MBC (Anderson and Domsch, 1990).

3 A correlation analysis was performed in order to identify the relationships among
4 the evaluated properties. Then Principal Components Analysis (PCA) was applied
5 using data of physical and chemical soil properties as well as MBC, CO_2 , qCO_2 ,
6 enzyme activities to determine the degree of association of the variables analyzed.
7 The number of components was determined by the eigenvalue-one criterion.
8 Subsequently, a hierarchical cluster analysis (Ward's method) was performed
9 using the software STATGRAPHICS 5.1.

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12 **3 Results**

13 **3.1 Soil physic-chemical properties**

14

15 Gravimetric moisture content ranged from 1.37 % (0 year old) to 2.81 % (15 year
16 old) and were correlated with the water holding capacities ($r=0.74$; $p<0.01$). The pH
17 values found ranged between 4.9 and 6.3 on mine soil. WHC values ranged from
18 27 g and 35 g 100 g^{-1} in mine soil amount representing 87 % of the value found in
19 undisturbed soil. The SOC level was found higher in undisturbed soil than mine soil
20 (Table 1). TN and SOC increased at a slower rate, in soil mine TN found came to
21 represent 59 %, while SOC only the 26 % than in undisturbed soil.

22

23 **3.2 Microbial properties**

24

25 The highest value of MBC was found in the undisturbed soil, which coincides with
26 the highest value of organic matter with respect to the mine soils (Table 2). After
27 extraction of pumice MBC content, S_0 decreases $> 50\%$ respecting to the S. The
28 highest values in the microbial properties were obtained in undisturbed soil, while
29 the rest of soils show similar values. This result indicated that the land degradation
30 is associated to a strong decrease in the SOC content which lowered to 76 % in

1 recently mine soils with respect to undisturbed soil. The respiration rate was higher
2 in undisturbed soils than in another treatments.

3 Enzyme activities rise with increasing age of mined: undisturbed soil showed the
4 highest enzyme activity values, while the mine soil the lowest (Table 2). In this
5 study, urease activity in undisturbed soils was particularly high, recovered quickly
6 on mined sites; 15 years after reclamation the urease activity levels had reached
7 more than 87 %

8 The qCO_2 values were higher in mine soils compared to the undisturbed soils
9 (Table 2), meaning that the microorganisms were more efficient in conserving C
10 resources in the no mined sites as compared than in the mined sites.

11

12 **3.3 Correlations**

13 Correlation matrix revealed a negative correlation of qCO_2 , with MBC, WHC,
14 catalase and GMC. Closer correlations were found among chemical and biological
15 variables; the MBC was positively correlated with WHC while correlations among
16 catalase and urease activity were found. Additionally, both MBC and WHC were
17 positively correlated with TN (Table 3).

18 Principal components analysis (PCA) was performed using all the data for soil
19 characteristics to explore the differences between soils; the main results are shown
20 in Table 4. The weighted loading values in each component were used to select
21 the indicators, with ten percent of the highest weighted loading as a threshold for
22 selection (Govaerts et al., 2006). The first component accounted for 33.27 % of the
23 total variance and the highest loadings were found with TN followed by MBC and
24 pH. Contribution from the second component of the total variance was 23.97 %
25 with the highest loading was found with catalase and urease. PC3 accounted for
26 14.74 % of the total variance and was mainly associated with EC and P. According
27 to the PCA results pH and TN were the most important characteristics that affected
28 MBC.

29

30 **3.5 Cluster analysis**

31

1 Cluster analysis is often used to determine group associations and to assess the
2 affinity among different variables (Hussain et al., 2008). The dendrogram obtained
3 using Euclidean distance as similarity criterion (Fig. 1) revealed a clear separation
4 into two major groups. The first cluster was found S_{10} , S_{15} and S_0 , being greater the
5 similarity between the soil with more years of mining (15) and undisturbed soil. The
6 second group was formed by soils with shorter mined (S_0 , S_1 and S_4), finding
7 greater similarity between S_1 and S_4 .

8 In the present study, Dendrogram Analysis (DA) was applied to differentiate soils
9 with different age of mined as well as to determine whether the difference was
10 actually significant or not. Three major soil clusters were evident. Canonical scores
11 indicate that S_{15} reclamation site is more similar to the S_{10} site than that of the
12 others mined sites (Fig. 2). Although DA was clearly able to discriminate between
13 disturbed and undisturbed ecosystems, indicated a trend towards the undisturbed
14 condition with reclamation age. The graph showed two major groups, the first one
15 consists of the three most disturbed soils (S_0 , S_1 and S_4) and the second group
16 corresponds to the two least disturbed soils (S_{15} and S_{10}).

17

18

19 **4 Discussion**

20

21 The loss of SOC and TN observed in soils post-pumice mining is typical of major
22 ecosystem disturbance (Kimble et al., 2001). The depletion of organic C produces
23 a decline in soil quality associated with to the reduction of available water capacity,
24 nutrient concentration and soil structure (Schwenke et al., 2000).

25 Our results also indicate that disturbance was highly detrimental in SOC, only 45
26 % of amounts found for the undisturbed site. As result of direct photodegradation
27 by solar radiation (Austin and Vivanco, 2006) as well as the mixing of surface soil
28 with deeper soil layers (Ward, 2000). The quantity of organic matter (C and N) and
29 rates of microbial C mineralization to CO_2 (respiration) were recovered with age in
30 mining soil. Shrestha and Lal (2011), aimed to quantifying the effects of mining and
31 reclamation processes on physical and chemical properties of reclaimed soils for

1 three dominant soil series in Ohio. Mining and reclamation activities decreased
2 (SOC) declined by 52 to 83 % of undisturbed sites and nitrogen (N) pools declined
3 from 42 to 75 % of undisturbed sites.

4 The recovery of MBC in these soils was faster than organic C. This may indicate
5 that the proportion of bio-available C in 15-year-old soils has become similar to that
6 of undisturbed soils but may also be the result of an increased availability of TN
7 (evidenced by lower C/N ratios in older soils) and inorganic P (as a result of
8 fertilization). The MBC is greatly reduced from the undisturbed soil in the mine soil
9 is estimated an average of only 76%. The results obtained by Insam et al. (1991)
10 and Rodriguez et al. (2005) demonstrated the relationship between MBC and soil
11 TN.

12 The increase in soil basal respiration in mine soil can be explained by an
13 increase in the contents of SOC and nutrients, which would enhance microbial
14 activity (Emmerling et al., 2000), and biomass cycling, thus leading to an increase
15 in basal respiration (Fernandes et al., 2005).

16 To estimate the activity and efficiency of decomposition (or C use) by the soil
17 microbes the qCO_2 was calculated (Anderson and Domsch, 1990). That
18 establishes, as the MBC becomes more efficient in using the available resources,
19 less C is lost as CO_2 through respiration (García-Orenes et al., 2010). Soil
20 microbial quotients in several ecosystems have been found to increase
21 immediately post-disturbance and subsequently decline with age (Insam and
22 Domsch, 1988; Schipper et al., 2001; Graham and Haynes, 2004). This pattern of
23 microbial quotient has been interpreted as indicative of a decrease in C bio-
24 availability in the soil organic matter over time. The microbial quotient of 0-year-old
25 was higher than other mine soils and it trend to increased with age. The respiration
26 rate per unit of microbial biomass (respiratory quotient) is a variable that can be
27 interpreted easier (Fernandes et al., 2005). The lower qCO_2 values at S site may
28 suggest that is a more stable ecosystem with high levels of biological activity. The
29 qCO_2 is a valid indicator of the efficiency of energy use by microbes (Yan et al.,
30 2003). High qCO_2 values suggest that the soil microbes are competing intensely
31 for the small amount of C available.

1 The principal components analysis indicated that TN, MBC followed similar an
2 accumulating trend and, after 15 years of restoration point to successful recovery
3 of the state conditions reference.

4 5 6 **5 Conclusions**

7
8 This study evidenced that pumicite extraction has had an adverse impact
9 on the quality of soils cultivated with maize. What is most evident in the first
10 years after the mine; after several cycles of cultivation and recovered MBC,
11 increased enzyme activities, apparently as a result of the continuous additions
12 of organic matter.

13 Using multivariate analysis provided information about soil indicators that
14 contributed to a greater extent to determine the effects of extraction pumicite.
15 Among the evaluated quality indicators soil, microbial biomass carbon stocks
16 OC and TN, highlighted in the multivariate analysis and provided information to
17 diagnose the quality of the soil where pumicite was removed.

18 19 20 **6 References**

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Table 1. Chemical and physical properties of soil samples with different years of extraction of pumice.

Sample		GMC	WHC	pH 1:2.5	EC	BD	P	TN	SOC	C/N
		(g·100g ⁻¹)		(soil:H ₂ O)	(dS m ⁻¹)	(g cm ⁻³)	(mg kg ⁻¹)	(g·100g ⁻¹)		
S ₀	February	2.33±0.11	31±0.57	5.5±0.31	0.13±0.00	1.18±0.01	16.23±0.19	0.08±0.03	1.30±0.12	18 ±5.30
	June	2.27±0.11	29±0.58	5.9±0.16	0.17±0.00	1.17±0.02	16.23±0.14	0.11±0.00	0.72±0.11	7±1.00
	March	1.37±0.09	30±0.58	6±0.08	0.16±0.01	1.12±0.01	16.91±0.14	0.10±0.01	1.20±0.05	12±2.12
S ₁	February	2.48±0.13	33±2.08	5.1±0.30	0.08±0.00	1.21±0.01	16.23±0.03	0.11±0.0	1.23±0.11	11±1.05
	June	2.40±0.01	30±0.58	6.2±0.16	0.35±0.01	1.15±0.01	17.80±0.01	0.11±0.00	0.84±0.11	8±1.05
	March	1.60±0.17	30±0.00	6.2±0.01	0.17±0.01	1.12±0.01	17.02±0.26	0.11±0.00	1.30±0.05	12±0.42
S ₄	February	2.59±0.03	35±0.00	5.2±0.04	0.07±0.00	1.21±0.00	16.72±0.20	0.17±0.00	1.50±0.11	9±0.65
	June	2.63±0.05	30±0.00	6.2±0.56	0.30±0.02	1.2±0.04	17.80±0.07	0.11±0.00	0.72±0.11	7±1.00
	March	1.67±0.02	30±0.00	6.3±0.04	0.21±0.00	1.2±0.02	17.57±0.42	0.12±0.02	1.25±0.08	11±1.00
S ₁₀	February	1.68±0.02	30±0.00	4.9±0.14	0.10±0.00	1.31±0.00	21.95±0.64	0.19±0.03	1.56±0.20	8±0.66
	June	1.71±0.04	27±0.00	5.4±0.20	0.45±0.11	1.20±0.03	20.71±0.07	0.17±0.00	1.60±0.20	9±1.15
	March	1.289±0.25	27±0.00	5.5±0.03	0.27±0.00	1.24±0.02	23.93±0.49	0.17±0.00	1.50±0.12	9±0.72
S ₁₅	February	2.14±0.02	34±0.58	5.1±0.09	0.18±0.01	1.23±0.04	18.89±0.19	0.17±0.00	1.63±0.23	10±1.32
	June	2.81±0.06	31±0.58	5.4±0.10	0.47±0.01	1.10±0.01	20.43±0.13	0.11±0.00	0.78±0.00	7±0.00
	March	1.80±0.37	29±0.58	6.0±0.05	0.19±0.00	1.10±0.00	19.07±0.13	0.11±0.00	1.09±0.08	10±0.73
S	February	3.16±0.07	40±3.78	4.7±0.18	0.10±0.00	1.11±0.018	16.77±0.19	0.32±0.03	2.28±0.11	7±1.00
	June	3.11±0.02	34±0.58	5.6±0.14	0.13±0.00	1.02±0.01	15.74±0.05	0.23±0.02	2.67±0.11	12±0.75
	March	2.15±0.10	33±0.58	5.4±0.11	0.24±0.00	1.02±0.03	16.68±0.12	0.19±0.00	2.73±0.00	14±0.00

GMC: Gravimetric moisture content, WHC: water holding capacity, EC: electrical conductivity, BD: bulk density; P: available phosphorus, TN: total nitrogen, SOC: soil organic carbon, S₀: recently mined, S₁: one year old mined, S₄: 4 years old mined, S₁₀:10 old years mined, S₁₅: 15 years old mined, S: undisturbed soils. mean ± standard deviation.

Table 2. Enzymatic activity and soil microbial properties of soil samples with different years of extraction of pumice.

Sample		Urease		Phosphatase		Catalase		MBC	CO ₂	qCO ₂
		($\mu\text{moles N-NH}_4^+ \text{ g}^{-1} \text{ h}^{-1}$)	Specific activity	($\text{mmoles: PNP g}^{-1} \text{ h}^{-1}$)	Specific activity	($\text{mmoles H}_2\text{O}_2 \text{ consumed g}^{-1} \text{ h}^{-1}$)	Specific activity	MBC (mg C kg s^{-1})	($\text{mg C-CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$)	
S ₀	February	5.33±1.15	5.52	2.70±0.26	4.25	0.11±0.06	2.11	246±28	1847±49	2.6±0.34
	June	6.67±0.58	12.07	2.14±0.07	9.68	0.12±0.01	3.04	134±25	837±39	3.1±1.25
	March	11.33±1.16	14.69	1.36±0.05	9.57	0.26±0.01	1.17	147±18	1669±40	4.4±1.27
S ₁	February	7.00±1.00	8.22	2.60±0.04	5.90	0.09±0.02	2.13	284±98	1849±22	2.2±0.15
	June	10.67±0.58	16.14	2.01±0.13	1.02	0.14±0.02	2.43	150±19	861±00	2.0±0.23
	March	12.67±1.16	13.37	1.40 ±0.09	9.83	0.29±0.05	1.10	166±20	1804±16	3.8±0.40
S ₄	February									
	June	9.67±0.58	7.87	1.12±0.01	6.61	0.11±0.06	0.76	313±29	1849±23	2.1±0.17
	March	6.33±1.15	11.01	2.76±0.42	9.41	0.16±0.03	3.95	150±19	862±00	2.0±0.23
		13.00±0.00	14.41	1.35±0.15	10.56	0.28±0.05	1.10	199±18	1749±55	3.3±0.60
S ₁₀	February									
	June	10.33±0.58	9.93	2.91±0.07	7.07	0.14±0.04	1.90	348±19	1904±28	1.9±0.10
	March	4.00±1.00	6.94	3.17±0.21	2.88	0.13±0.02	2.07	115±12	899±00	4.1±1.38
S ₁₅	February									
	June	11.67±1.52	9.15	3.07±0.04	7.47	0.20±0.01	1.92	388±9	1985±21	1.7±0.05
	March	10.33±1.53	15.59	3.18±0.06	13.71	0.15±0.02	4.09	117±18	903±00	2.8±0.46
		14.00±0.58	13.82	1.88±0.09	13.12	0.34±0.01	1.76	230±18	2240±19	3.6±0.50
S	February									
	June	11.00±1.00	6.73	2.67±0.09	5.23	0.23±0.05	1.19	458±42	2146±66	1.6±0.20
	March	8.34±2.10	3.53	3.68±0.04	3.47	0.12±0.01	1.40	259±32	966±13	1.3±0.15
	February	16.00±1.73	8.93	2.17±0.05	6.02	0.35±0.00	0.82	248±40	2369±60	3.4±0.75

MBC: microbial biomass C, CO₂: respiratory activity, qCO₂: metabolic quotient, S₀: recently mined, S₁: one year old mined, S₄: 4 years old mined, S₁₀: 10 old years mined, S₁₅: 15 years old mined, S: undisturbed soils. mean ± standard deviation.

1 **Table 3.** Correlation matrix between the different parameters determined.

Parameter	EC	Catalase	MBC	SOC	CO ₂	WHC	GMC	TN	pH	qCO ₂	urease
EC	1										
Catalase	NS	1									
MBC	-0.67	NS	1								
SOC	NS	NS	0.53	1							
CO ₂	-0.58	0.67	0.64	NS	1						
WHC	NS	NS	0.75	NS	NS	1					
GMC	NS	NS	NS	NS	NS	0.74	1				
TN	NS	NS	0.68	0.78	NS	0.64	NS	1			
pH	NS	NS	-0.76	NS	NS	NS	NS	-0.64	1		
qCO ₂	NS	-0.77	-0.61	NS	NS	-0.66	-0.77	NS	NS	1	
Urease	NS	0.84	NS	NS	0.60	NS	NS	NS	NS	NS	1

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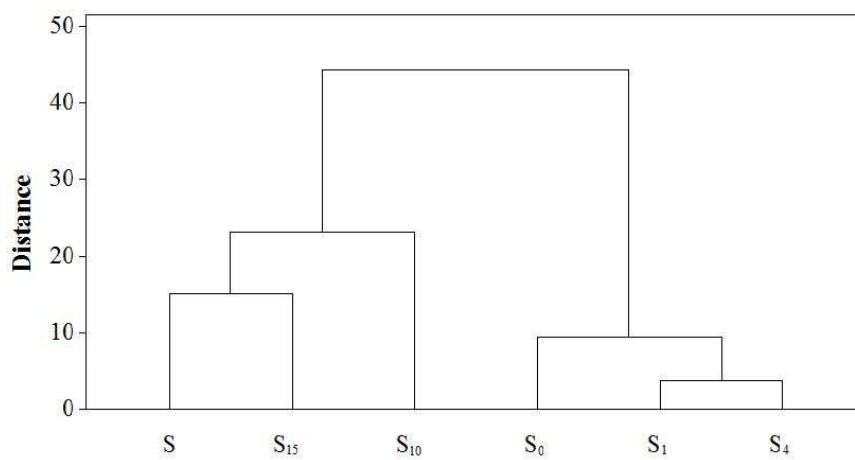
3 EC: electrical conductivity, CO₂: activity respiratory, WHC: water holding capacity, GMC: gravimetric moisture
4 content, TN: total nitrogen, qCO₂: metabolic quotient, NS: not significant, p < 0.01.

1 **Table 4.** Results of principal component analysis.

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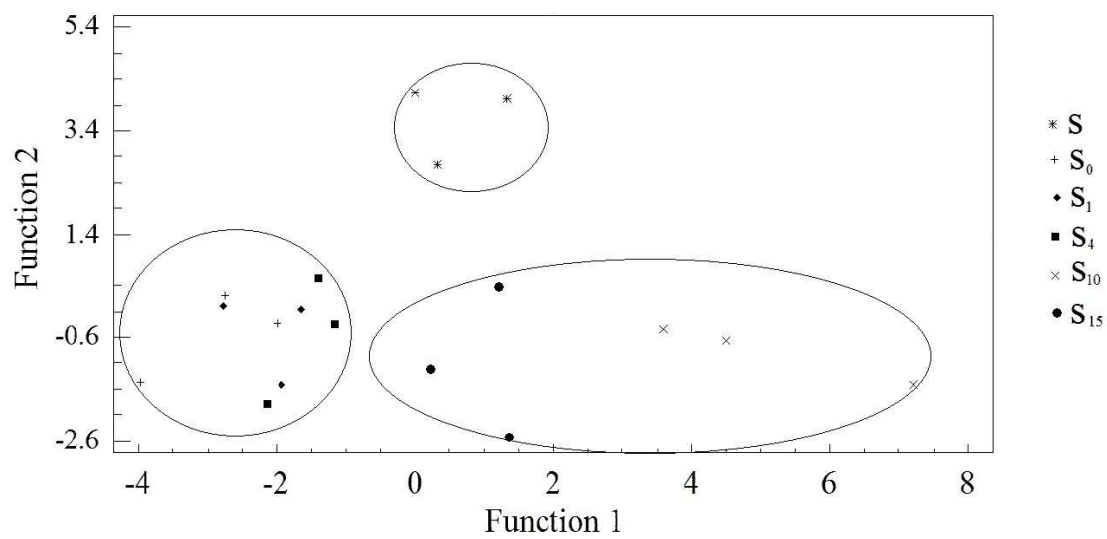
Variable	Component				
	1	2	3	4	5
GMC	0.371	-0.513	-0.464	0.451	-0.337
WHC	0.634	-0.037	-0.655	0.271	-0.132
pH	-0.864	0.151	0.081	0.303	-0.040
EC	-0.258	-0.204	0.777	0.259	-0.307
Bulk density	-0.019	-0.125	0.055	-0.957	-0.130
MBC	0.803	0.181	-0.483	-0.243	0.016
TN	0.880	0.127	-0.042	0.184	-0.193
SOC	0.762	0.193	-0.043	0.386	0.353
C/N	-0.035	0.050	-0.158	0.150	0.942
Urease	0.096	0.891	-0.043	0.235	-0.064
Phosphatase	0.523	-0.694	0.240	0.173	-0.018
Catalase	0.090	0.938	0.228	0.083	0.072
P	0.147	0.143	0.785	-0.481	-0.221
CO ₂	0.392	0.707	-0.225	-0.269	0.397
qCO ₂	-0.512	0.418	0.517	0.018	0.358
Variation	33.276	23.976	14.475	11.046	7.914
Cumulative variation	33.276	57.252	71.727	82.773	90.687

3 Bold values indicates the higher weight variables are considered highly weighted within 10 % of variation of the
4 absolute values of the highest factor loading in each PC. GMC: gravimetric moisture content, WHC: water
5 holding capacity, EC: electrical conductivity, MBC: microbial biomass C, TN: total nitrogen, SOC: soil organic
6 carbon, P: available phosphorous, CO₂: activity respiratory, qCO₂: the metabolic quotient.



1 **Figure 1.** Dendrogram obtained (Ward method) for cluster analysis of different age mined and
 2 undisturbed soil. S₀: recently mine, S₁: one year old mine, S₄: 4 years old mine, S₁₀:10 old years
 3 mine, S₁₅: 15 years old mine, S: undisturbed soils.

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4 **Figure 2.** Plot of canonical scores calculated by discriminant analysis for the different age of mine
 5 soil. S₀: recently mined, S₁: one year old mine, S₄: 4 years old mine, S₁₀:10 old years mine, S₁₅: 15
 6 years old mine, S: undisturbed soils.