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# Improving the mining soil quality for a vegetation cover after addition of sewage sludges: Inorganic ions and low-molecular-weight organic acids in the soil solution

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**Abstract.** We assessed the effects of applying stabilized sewage sludge (SSL) and composted sewage sludge (CLV), at 5 and 10% to an acid mining soil. Limed soil (NCL) amended or not with SSL and CLV was incubated for 47 days. We studied the cations and organic and inorganic anions in the soil solution by means of ion chromatography. Liming led to big increases in  $\text{Ca}^{2+}$  and  $\text{SO}_4^{2-}$  and to significant decreases in  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Addition of both organic amendments increased some cations ( $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$  only with CLV and  $\text{PO}_4^{3-}$  only with SSL) and provided a greater amount of low-molecular-weight organic acids (LMWOAs) (SSL more than CLV). Incubation led to decreases in all cations, particularly remarkable for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in SSL-10. A decrease in  $\text{NH}_4^+$  was associated with variations in  $\text{NO}_2^-$  and  $\text{NO}_3^-$  resulting from nitrification reactions. During incubation the LMWOAs content tended to decrease similarly to the cations, especially in SSL-10. Chemometric tools revealed a clear discrimination between SSL, CLV and NCL. Furthermore, treatment effects depended upon dose, mainly in SSL. Amendment nature and dose affect the quality of a mining soil and improve conditions for plant establishment.

**Keywords:** Mining soil, Liming, Organic amendments, Organic acids, Ion chromatography, Multivariate analyses

## 1. Introduction

Mining areas constitute degraded ecosystems resulting from mineral extraction for over long periods of time giving rise to large areas of derelict land. Intensification of mining causes an ecological impact at local and regional scales. Mining wastes can be stabilised by revegetation policies (Clemente et al., 2007). Establishment of a vegetation cover calls for a water-soil system capable of supporting the nutrient and water requirements of plants. In acid mine tailings, revegetation must primarily overcome extreme acidity by addition of a liming agent, and then correct nutrient deficiencies by application of fertilizers.

In Andalusia (Southern Spain) mining activities date from pre-Roman times. Intensive deforestation has led to degraded and abandoned land incapable of sustaining commercial agriculture, as well as destruction of the natural vegetation. In the Riotinto mining area, located in the Iberian Pyrite Belt, acidification of soils occurred due to oxidation of the metallic sulphides (pyrite) transported by acidic mine drainage and to atmospheric deposition of wind-blown dust (Rodríguez-Liébana et al., 2013). These soils present high levels of toxic elements and low levels of plant nutrients; so, application of organic residues has become a popular strategy due to the agronomic benefits involved. In addition, although the total levels of toxic elements is high, concentrations of soluble and exchangeable chemical forms of metals depend on their bio-accessibility properties and speciation, being those levels usually lower than total concentrations (Ciarkowska et al., 2014). For instance, Martínez-Sánchez et al. (2013) demonstrated that arsenic bio-accessibility to humans in mine soils was really low regarding the high total concentrations. Besides, speciation studies next to mine soils have shown that there was no risk to humans by soluble lead and zinc levels in arable and pasture lands (Rodríguez et al., 2009). However, the possible negative effects in plants depend on the metal studied; this way, Struckhoff et al. (2013) showed that high concentrations of lead caused adverse native floristic quality, although no other human disturbance was evident.

*Abbreviations:* CA, Cluster Analysis; CLV, Compost of sewage sludge with vegetal remains; DOC, Dissolved organic carbon; LMWOAs, Low-molecular-weight organic acids; LOD, Limit of detection; LOQ, Limit of quantitation; NC, Native soil; NCL, Limed soil, with  $\text{CaCO}_3$  (Carbocal); PCA, Principal Component Analysis; PCs, Principal components; RSD, Relative standard deviation; SSL, Stabilized sewage sludge;  $t_{1/2V}$ , Time for the disappearance of half of the initial ion concentration;  $t_{1/2I}$ , Time for the increase to 50% the initial ion concentration.

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Urban sewage sludge constitutes an economical alternative and contributes to solving the problem of waste disposal. Furthermore, composted residues have become an attractive option, because degradation-transformation reactions during the composting process turn the organic matter into a more stable humus-like product (Senesi, 1989). Organic amendments improve soil quality but, at the same time, supply the soil solution with organic and inorganic ions (Kayikcioglu, 2012). Bioremediation of soils with biochars, where carbon mineralization is lower and slow, constitutes another alternative to the conventional amendments (Wuet al., 2014; Sun et al., 2014). Although the benefits of lime and organic residues addition are initially evident, the incubation process leads to changes in soil solution composition, with possible implications for retention or availability of nutrients or other compounds (Rodríguez-Liébana et al., 2011). Not only the inorganic composition of soil solution will evolve with waste addition, but also the content in low- molecular-weight organic acids (LMWOAs) (Drever and Stillings, 1997). LMWOAs have been reported to play key roles in many soil and plant processes, relying on their ability to complex metal cations (Jones et al., 2003; Strobel, 2001) or to interfere in sorption-desorption processes of hydrophobic organic pollutants (González et al., 2010). Concentrations in non-amended soil solution of aliphatic mono-, di- and tri-carboxylic acids are an order of magnitude lower than those of inorganic solutes. Furthermore, application of organic amendments implies the introduction of large amounts of dissolved organic matter, with carbon in LMWOAs comprising less than 10% of total dissolved organic carbon (DOC) in most soil solutions (Strobel et al., 1999). We therefore attempted to evaluate the changes in the soil- solution composition of an acidic mine soil amended with organic residues from urban sewage sludge. An understanding of the chemistry of the soil-water-plant interface is important with regard to optimising revegetation strategies (Mingorance et al., 2014). A vegetation cover improves soil quality by reducing soil erosion, both eolian and water erosion, by improving soil structure and natural soil fertility and by favoring microbial activity. In addition, the application of a vegetative cover helps to avoid the natural dispersion of pollutants along the time (Rodríguez et al., 2009; Vázquez et al., 2011). Although this study was not focused directly on vegetation cover, it takes part of a more wide study aiming at improving soil quality with a view to revegetating mine soils (Rodríguez-Liébana et al., 2013; Mingorance et al., 2014; Peña et al., 2014).

## 2. Experimental methods

### 2.1. Site description. Soil and amendment properties

We collected the soil (NC) from an abandoned mine in the Riotinto mining zone, in the vicinity of the village of Nerva, Huelva province (SW Spain) (Fig. 1), located in the Iberian Pyrite Belt. Soil collection and characterization was done as in Mingorance et al. (2014). In brief, the sandy-loam soil (55% sand, 14% clay) is extremely acidic ( $\text{pH}_{\text{H}_2\text{O}}$  2.4), presents high electrical conductivity ( $1.3 \text{ dS m}^{-1}$ ) (both at 1:2.5 ratio) and low organic carbon content (1.4%). The content of some potentially hazardous metals and non-metals usually present in mining areas (As, 3951; Cd, 13; Cu, 694; Pb, 3976 in  $\text{mg kg}^{-1}$ ) is above local guidelines for total concentrations (Aguilar et al., 1999).

We employed two organic amendments from wastewater treatment plants: stabilized sewage sludge (SSL) and composted sewage sludge with remains from olive pruning (CLV). Both SSL and CLV share, respectively, a neutral pH (6.9 and 7.0), have a humification index (HIX) of 0.43 and 2.16, as well as high OC content (35.5 and 16.0%) and electrical conductivity ( $2.8$  and  $4.2 \text{ dS m}^{-1}$ ) (1:10 ratio). Humic acid content was 1.6 and 8.2% and fulvic acid content, 0.47 and 7%, respectively, for SSL and CLV. The potential toxic metal loads of SSL at the 5% dose and CLV at any dose were lower than those corresponding to European guidelines concerning external application to soil (European Commission, 1986).

### 2.2. Incubation procedure

Prior to addition of amendments, soil was first limed ( $\text{NC}_L$ ) with Carbocal (Azucarera Ebro), a residue rich in  $\text{CaCO}_3$  (83.4% and an OC content of 5.1%) at an equivalent rate of 1.5% (w/w) calcium carbonate e  $0.75 \text{ g kg}^{-1}$  e to raise soil pH to physiological values. The organic wastes were then added at 5 and 10% (w/w), corresponding to approximately 100 and 200  $\text{Mg Ha}^{-1}$ . The mixtures, consisting of air-dried soil and amendments, were placed in plastic trays covered with aluminium foil to avoid desiccation; their moisture was adjusted to 40% of the soil field capacity with deionised water, and they were left at room temperature ( $20 \pm 2 \text{ }^\circ\text{C}$ ) for up to 47 days, with periodical water irrigation, as required, to maintain soil humidity.

After 12, 29 and 47 days of incubation, subsamples were taken from each tray and frozen prior to measure ion concentrations in soil solution. Prior to sampling, we thoroughly mixed each soil sample to ensure homogeneity and representativeness.

### 2.3. Extraction and analysis. Instrumental determinations

Based on the evaluation of sequential extraction schemes for soils, sediments (Nyamangara, 1998) and other matrices (Smichowski et al., 2008), and assessing their first fractions, we employed water as the extractant for the soil solution, particularly recommended in studies of organic acids (Senesi, 1989; Piombo et al., 1996). We extracted soluble ions by shaking soils with ultrapure (Milli-Q) water (1:10 ratio) for 1 min, using a vortex shaker; we then centrifuged at 4000 rpm for 5 min and decanted. Following a 1:10 dilution, 1 mL of solution was simultaneously injected and filtered (0.2 mm  $\varnothing$ ) into the chromatographic system.

Following extractions, we performed determinations of all ionic compounds in 18 samples with the use of an isocratic high-efficiency Ion Chromatography-Conductivity Detector (IC-CD) (Metrohm AG, 792 Basic IC, Herisau, Switzerland) with the Metrohm Chemical Suppression Module to increase sensitivity in the anion response. We determined nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ), minor and major cations ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ) and organic (formate- $\text{CHO}_2^-$ , acetate- $\text{C}_2\text{H}_3\text{O}_2^-$ , oxalate- $\text{C}_2\text{O}_4^{2-}$ , phthalate- $\text{C}_8\text{H}_4\text{O}_4$ ) and inorganic ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{I}^-$ ) anions; the experimental conditions were reported by Montoya-Mayor et al. (2011). All determinations in the multielemental analyses were performed at least in triplicate ( $n = 3-4$ ) for each sample.

### 2.4. Method performance. Linearity and precision studies of calibration curves

Calibration curves were constructed with the use of concentration ranges similar to the level of ions in real samples. We employed Fluka certified standards at 1000 ppm -TraceCERT<sup>®</sup>- traceable to NIST SRM (Sigma-Aldrich) to prepare the calibration points, by appropriate dilution of the stock standard with the same eluent. Calibration results (Table 1) show that curves were linear (Cuadros et al., 1996) for all ions (>97.8%) for wide linear ranges 99.8% for 0-50,000 ng mL<sup>-1</sup> (ppb) of sodium and 99.5% for 0-40,000 ng mL<sup>-1</sup> of sulfate-, as well as for small linear ranges 98.8% for 0-100 ng mL<sup>-1</sup> of formate and 98.2% for 0-200 ng mL<sup>-1</sup> of bromide. We analysed each calibration point six times over six non-consecutive days; RSD values were lower than 3% for all analytes, even for ions at low trace concentrations in samples, such as formate, phosphate or bromide. Global RSD values were within the range of 0.72e3.11% for the six non-consecutive days.

### 2.5. Limits of detection (LOD) and quantitation (LOQ)

We calculated the limits of detection and quantitation (LOD and LOQ) as the concentration corresponding to a signal 3 and 10 times the standard deviation of the intercept of the calibration curve, respectively. The chemical suppression module in the chromatograph reduced the habitual LOD values (Table 1), such as from 0.40 ng mL<sup>-1</sup> for nitrite to 6.6 ng mL<sup>-1</sup> for bromide. In the case of cations low LOD values were also achieved (from 11.9 ng mL<sup>-1</sup> for potassium to 126 ng mL<sup>-1</sup> for sodium). We have determined standards of four organic acids with the inorganic anion column; with low detection limits, below 5 ng mL<sup>-1</sup>. The ions most concentrated in the real samples, sulfate and oxalate, were those with higher values of LODs. We have measured standards of iodine, strontium and barium as the ions running at high retention time values; they therefore also shared higher LOD and LOQ values.

### 2.6 Accuracy studies with certified reference material

The accuracy of the chromatographic determinations was established by the international Certified Reference Materials BCR-409 supplied by the Institute for Reference Materials and Measurements (Geel, Belgium) from 18 replicates over six non-consecutive days, that is to say, six inter-day replicates averaged for three intra-day replicates. The recoveries for all elements ranged from 90.3 to 110% with values within the range of the Association of Analytical Communities (AOAC, 2002) (Table 2). The t-test showed that there were no significant differences between experimental averages and theoretical values. The Fisher-Snedecor test showed that the experimental standard deviations were similar to the typical deviations of the BCR 409 for all analytes certified: values of  $F_{\text{calculated}}$  varied from 1.03 to 2.56, while the  $F_{\text{tabulated}}$  value range was 2.57e3.00. The precision values, defined as the relative standard deviation (%RSD), therefore varied from 0.94% for sulfate to 3.4% for magnesium, and were in accordance with those obtained from the Horwitz-1982 function depending on the analyte level (Worwitz, 1982). These RSD values were higher than those obtained with certified standards used in regression curves (Table 1). The  $\text{RSD}_{\text{inter-day}}$  and  $\text{RSD}_{\text{intra-day}}$  were below 3% for all analytes, suggesting that the protocol and the system were quite steady. However, the  $\text{RSD}_{\text{inter-day}}$  was not always worse than the  $\text{RSD}_{\text{intra-day}}$ , likely due to the different response to fluctuation of the system during the all-day operation.

**Table 1**

Calibration parameters of the IC-CD analysis (LOD: limit of detection, LOQ: limit of quantitation).

Analyte	Linearity (%)	RSD <sub>g</sub>	LOD	LOQ	Linear range
		(%, n ¼ 6)	(ng mL <sup>-1</sup> /mM)	(ng mL <sup>-1</sup> /mM)	(ng mL <sup>-1</sup> )
<b>Anions</b>					
F <sup>-</sup>	99.3	2.13	2.6/0.14	8.6/0.45	0 ~ 200
Cl <sup>-</sup>	99.7	0.91	9.7/0.27	32/0.90	0 ~ 2000
NO <sub>2</sub> <sup>-</sup>	100	1.73	0.36/0.008	1.2/0.03	0 ~ 2000
Br <sup>-</sup>	98.2	1.66	6.6/0.08	22/0.28	0 ~ 200
NO <sub>3</sub> <sup>-</sup>	99.7	1.11	12/0.19	39/0.63	0 ~ 5000
PO <sub>4</sub> <sup>3-</sup>	99.5	2.05	4.7/0.05	16/0.17	0 ~ 500
SO <sub>4</sub> <sup>2-</sup>	99.5	0.72	360/3.8	1200/12	0 ~ 40000
I <sup>-</sup>	98.2	2.87	54/0.42	180/1.4	0 ~ 2000
<b>Cations</b>					
Li <sup>+</sup>	99.5	2.22	41/5.9	138/20	0 ~ 5000
Na <sup>+</sup>	99.8	0.87	126/5.5	422/18	0 ~ 50,000
NH <sub>4</sub> <sup>+</sup>	100	0.83	13/0.72	43/2.4	0 ~ 20000
K <sup>+</sup>	99.7	1.60	12/0.31	40/1.0	0 ~ 2000
Ca <sup>2+</sup>	99.5	1.29	21/0.52	71/1.8	0 ~ 2000
Mg <sup>2+</sup>	97.8	1.96	76/3.1	252/10	0 ~ 2000
Sr <sup>2+</sup>	99.1	2.61	211/2.4	702/8.0	0 ~ 20000
Ba <sup>2+</sup>	99.5	3.11	143/1.0	476/3.5	0 ~ 20000
<b>Organic acids</b>					
C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	99.6	2.14	1.8/0.03	5.9/0.1	0 ~ 2000
CHO <sub>2</sub> <sup>-</sup>	98.8	1.36	2.0/0.04	6.6/0.15	0 ~ 100
C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	99.9	0.98	5.2/0.06	17/0.19	0 ~ 2000
C <sub>6</sub> H <sub>4</sub> O <sub>6</sub> <sup>3-</sup>	100	1.57	1.2/0.007	3.9/0.02	0 ~ 2000

RSD<sub>g</sub>(%): Global relative standard deviation in percentage for all the *n* inter-day replicates.

## 2.7 Data analysis

On the basis of the results, basic and multivariate statistical analyses were applied to the analytical data. ANOVA was used to detect significant differences ( $p < 0.05$ ) between amendments, doses and incubation days. The Post-Hoc Tukey's HSD test was also used in the ANOVA for comparison of several means. Principal Component Analysis (PCA) and Cluster Analysis (CA) were the statistical techniques employed as classification tools (Rossini-Oliva and Fernández-Espinoza, 2007). For the PCA, CA analyses and Pearson correlations ( $r$ ), the STATISTICA version 7 (2004) software package (StatSoft) was used. The PCA was performed with varimax rotation, extracting the factors or principal components (PCs).

Each selected factor should explain at least 5-10% of the total

variance of the dataset, and together they should account for over 75% of the accumulated variance. Subsequent to the PCA, we applied a Hierarchical Cluster Analysis to the standardized matrix of samples, using Ward's Method as the amalgamation rule and Euclidean distance as the metric.

**Table 2**

Results for measured cation and anion levels in the BCR-409 reference material.

Ion	Concentration obtained ± SD (µg mL <sup>-1</sup> ) (n = 18)	CRM concentration ± SD (µg mL <sup>-1</sup> ) (N)	Recovery (%)	AOAC criteria (%)	F <sub>calc</sub>	F <sub>tab</sub>	RSD <sub>ra</sub> (%), n = 3	RSD <sub>er</sub> (%), n = 6
Cl <sup>-</sup>	3.94 ± 0.08	4.01 ± 0.07 (20)	98.2	80-115	1.306	2.567	1.10	1.67
NO <sub>3</sub> <sup>-</sup>	4.86 ± 0.09	4.84 ± 0.06 (19)	100.4	80-115	2.250	2.617	1.27	1.81
SO <sub>4</sub> <sup>2-</sup>	5.09 ± 0.07	5.11 ± 0.07 (20)	99.6	80-115	1.030	2.633	0.94	1.29
Na <sup>+</sup>	1.85 ± 0.04	1.91 ± 0.03 (18)	96.9	80-115	1.778	2.673	1.42	1.09
NH <sub>4</sub> <sup>+</sup>	1.86 ± 0.04	1.91 ± 0.04 (14)	97.4	80-115	1.494	3.004	1.68	1.11
K <sup>+</sup>	0.183 ± 0.007	0.166 ± 0.008 (12)	110.2	75-120	1.306	2.870	1.90	3.07
Ca <sup>2+</sup>	0.561 ± 0.017	0.621 ± 0.012 (18)	90.3	75-120	2.007	2.673	1.94	2.54
Mg <sup>2+</sup>	0.298 ± 0.008	0.299 ± 0.005 (18)	99.7	75-120	2.560	2.673	1.57	3.35

SD: standard deviation; N: Number of accepted datasets

RSD<sub>ra</sub>(%): Intra-day relative standard deviation in percentage.

RSD<sub>er</sub>(%): Inter-day relative standard deviation in percentage.

## 3. Results and discussion

Concentrations of Li<sup>+</sup>, Sr<sup>2+</sup> and Ba<sup>2+</sup> were below LOD (41, 211 and 143 µg L<sup>-1</sup>) and those of F<sup>-</sup>, I<sup>-</sup> and Br<sup>-</sup> below LOQ (8.6, 180 and 22 µg L<sup>-1</sup>) in most samples; they were therefore omitted from the discussion. Table 3 shows concentrations of the 14 organic and inorganic ions in the 18 samples measured. Relative standard deviations oscillated from 0 to 21% depending on the ion and the type of amendment.

### 3.1. Addition of lime

Native and limed inorganic soil solution composition -non-amended soils- was dominated by the anions  $\text{SO}_4^{2-} \gg \text{NO}_3^- > \text{Cl}^-$ , and the cations  $\text{Ca}^{2+} \gg \text{Na}^+ > \text{Mg}^{2+}, \text{K}^+$ . With addition of lime,  $\text{Ca}^{2+}$  concentration obviously increased (16-fold,  $p = 0.014$ ) and due to the pH increase,  $\text{SO}_4^{2-}$  concentration was strongly enhanced (3-fold,  $p = 0.001$ ). Moreover, liming induced a decrease (3-4 fold) in some ions (Sharpley, 1991), such as  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Similar decreases in N (Hojito, 1998) or  $\text{Mg}^{2+}$  (Grove et al., 1981) concentration with liming have previously been reported.

The decrease in  $\text{K}^+$  has been attributed to the reduction of K saturation percentage of the cation exchange complex as a result of the lime-induced increase in cation exchange capacity (Barlett and McIntosh, 1969; Curtin and Smillie, 1995). Soil pH is an important factor influencing the solubility of P, which becomes more available at a pH value of 6.5-7.5 (Tan, 1994). Phosphate concentrations increased slightly with liming but not significantly ( $p = 0.202$ ) (Table 3). This increase is small because, despite the fact that phosphate has a high capacity for soil sorption (Jones and Brassington, 1998), the elevation of pH minimizes Al activity by transforming exchangeable Al to Al hydroxyl ions or precipitating Al hydroxides, and favours parallel production of OH and  $\text{PO}_4^{3-}$ , which compete for sorption sites in soils.

Consequently, the increase in pH with liming in the acidic soil would enable phosphorus uptake by plants to be enhanced due to the reduction of Al in the soil solution, although this increase in soluble P is ultimately dependent on soil mineralogy (Erich et al., 2002).

Regarding N species,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  decreased with liming three and four times, respectively ( $p = 0.036$  and  $0.010$ ), while  $\text{NO}_2^-$  levels showed a considerable increment ( $>6$ -fold,  $p = 0.045$ ). The nitrate decrease is due to a reduction to nitrite during iron sulphide oxidation induced by thiosulfate (not determined) (Haaijer, 2007) and stimulated by the increase of microbial activity during the first few days of incubation (Mingorance et al., 2014). The decrease in  $\text{NH}_4^+$  explained by the neutralization resulting from liming (carbonate). Similarly, in the initial stage of incubation, the mineralization of soil organic N and subsequent nitrification of  $\text{NH}_4^+$  might also explain the changes in N species and the increased soil pH (Yuan et al., 2011).

The composition of the organic acids shows that the concentrations of phthalic and acetic acids were not modified by liming ( $p = 0.408$  and  $0.275$ ), as expected for the weakly charged anion acetate (Jones and Brassington, 1998). But there is a remarkably big decrease in formate ion ( $\text{NC}_L/\text{NC} = 1/12$ ,  $p = 0.008$ ). This behaviour is explained by Zhang et al. (2004) as a consequence of photochemical reactions involving bicarbonate/formate binding. When the native acid soil -NC pH 2.4- is limed with Carbocal, carbonate is converted to bicarbonate. Bicarbonate ( $\text{HCO}_3^-$ ) and formate ( $\text{HCO}_2^-$ ) are known to share a similar structure and have similar charge delocalization.

Due to photochemical reactions, the bicarbonate ( $\text{CO}_2^{\bullet-}$ -radical anion, +IV) combines with formate (+II) to produce longer chain products, supporting a carbon-carbon coupling mechanism and competing with  $\text{HCO}_3^-$  for heterogeneous reaction sites, all of which decreases the source of formate.

The increase in oxalate ion in the soil solution (2-fold,  $p = 0.011$ ) might be due to the simultaneous occurrence of various opposing processes: lower sorption in soils as pH increases, precipitation of  $\text{Ca}^{2+}$ -oxalate in the presence of high concentrations of  $\text{Ca}^{2+}$  and an increase in free oxalate in soil solution with pH elevation (Violante and Gianfreda, 1993) resulting from the destruction of complexes with  $\text{Al}^{3+}$  stable at low pH (Cline et al., 1982).

In short, elevation of pH by liming favours uptake by plants of  $\text{Ca}^{2+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$  and oxalate, as well as retention-sorption by soils of  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and formate.

**Table 3**

Organic and inorganic ion content of native (NC), limed (NC<sub>L</sub>) and amended soils after addition of stabilized (SSL) and composted (CLV) sewage sludge, at 5 and 10%.

Sample/Dose (%)	Time (days)	Inorganic anions (mg kg <sup>-1</sup> )										Inorganic cations (mg kg <sup>-1</sup> )										Organic anions (mg kg <sup>-1</sup> )				
		Cl <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	CHO <sub>2</sub> <sup>-</sup>	C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	C <sub>6</sub> H <sub>4</sub> O <sub>4</sub> <sup>2-</sup>	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	CHO <sub>2</sub> <sup>-</sup>	C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	C <sub>6</sub> H <sub>4</sub> O <sub>4</sub> <sup>2-</sup>							
NC/0	12	4.90 ± 0.13	0.180 ± 0.005	65.1 ± 0.6	0.42 ± 0.03	1334 ± 65	26.1 ± 0.2	4.45 ± 0.07	26 ± 5	118 ± 17	25 ± 2	1.44 ± 0.06	1.87 ± 0.02	0.26 ± 0.02	0.29 ± 0.02	1.44 ± 0.06	1.87 ± 0.02	0.26 ± 0.02	0.29 ± 0.02							
NC/0	29	33.0 ± 0.2	0.275 ± 0.006	38.9 ± 0.2	0.65 ± 0.08	1422 ± 54	10 ± 2	4.0 ± 0.7	13.4 ± 0.4	102 ± 15	28.4 ± 1.1	1.16 ± 0.06	6.21 ± 0.10	0.43 ± 0.04	0.15 ± 0.02	1.16 ± 0.06	6.21 ± 0.10	0.43 ± 0.04	0.15 ± 0.02							
NC/0	47	23.99 ± 0.14	0.546 ± 0.006	48.00 ± 0.14	0.39 ± 0.07	1367 ± 54	11.9 ± 0.5	1.9 ± 0.2	6.7 ± 0.7	104 ± 5	23 ± 4	1.3 ± 0.2	9.3 ± 0.2	0.75 ± 0.07	0.41 ± 0.02	1.3 ± 0.2	9.3 ± 0.2	0.75 ± 0.07	0.41 ± 0.02							
NC <sub>L</sub> /0	12	16.33 ± 0.14	1.046 ± 0.008	13.3 ± 0.3	0.55 ± 0.09	3462 ± 68	31 ± 3	1.2 ± 0.2	6.3 ± 0.4	2176 ± 158	6.00 ± 0.09	1.6 ± 0.2	0.55 ± 0.10	1.02 ± 0.05	0.62 ± 0.02	1.36 ± 0.05	0.42 ± 0.02	0.49 ± 0.02	0.146 ± 0.005							
NC <sub>L</sub> /0	29	7.74 ± 0.04	1.643 ± 0.002	7.28 ± 0.06	0.76 ± 0.02	3312 ± 19	31 ± 4	1.0 ± 0.2	4.9 ± 0.3	965 ± 15	6.7 ± 0.4	1.36 ± 0.05	0.42 ± 0.02	0.49 ± 0.02	0.146 ± 0.005	1.34 ± 0.10	0.53 ± 0.07	1.32 ± 0.04	0.534 ± 0.009							
NC <sub>L</sub> /0	47	14.75 ± 0.07	2.776 ± 0.003	17.34 ± 0.09	0.62 ± 0.03	4224 ± 30	32 ± 6	0.7 ± 0.2	5.7 ± 0.8	2164 ± 96	7 ± 2	1.34 ± 0.10	0.53 ± 0.07	1.32 ± 0.04	0.534 ± 0.009	1.34 ± 0.10	0.53 ± 0.07	1.32 ± 0.04	0.534 ± 0.009							
NC <sub>L</sub> /NC	1/2															1/12	2		1.5							
NC <sub>L</sub> -CLV/5	12	56.9 ± 0.2	0.974 ± 0.009	232.8 ± 1.4	0.56 ± 0.11	3374 ± 30	55 ± 10	41 ± 5	30 ± 6	1749 ± 11	40 ± 2	2.2 ± 0.3	4.8 ± 0.2	0.39 ± 0.07	1.169 ± 0.008	2.2 ± 0.3	4.8 ± 0.2	0.39 ± 0.07	1.169 ± 0.008							
NC <sub>L</sub> -CLV/5	29	32.85 ± 0.14	2.050 ± 0.002	245.7 ± 1.4	0.54 ± 0.03	3318 ± 24	54 ± 4	36 ± 2	18 ± 2	1708 ± 3	23.5 ± 0.5	1.44 ± 0.07	3.62 ± 0.07	0.74 ± 0.03	1.050 ± 0.006	1.44 ± 0.07	3.62 ± 0.07	0.74 ± 0.03	1.050 ± 0.006							
NC <sub>L</sub> -CLV/5	47	89.8 ± 0.5	1.537 ± 0.008	233.8 ± 1.1	0.49 ± 0.10	3502 ± 77	59 ± 9	16 ± 3	10.3 ± 1.0	1971 ± 387	20 ± 3	1.8 ± 0.3	5.80 ± 0.14	1.10 ± 0.10	0.92 ± 0.03	1.8 ± 0.3	5.80 ± 0.14	1.10 ± 0.10	0.92 ± 0.03							
NC <sub>L</sub> -CLV/10	12	54.0 ± 0.3	1.318 ± 0.002	568.6 ± 1.0	0.46 ± 0.09	3459 ± 204	63.5 ± 1.3	89 ± 3	38 ± 3	1901 ± 203	51 ± 9	1.84 ± 0.06	7.41 ± 0.12	2.1 ± 0.2	1.2 ± 0.02	1.84 ± 0.06	7.41 ± 0.12	2.1 ± 0.2	1.2 ± 0.02							
NC <sub>L</sub> -CLV/10	29	61.5 ± 0.2	1.281 ± 0.002	595.3 ± 0.3	0.51 ± 0.02	3331 ± 28	72 ± 4	65 ± 4	35.7 ± 0.4	1790 ± 28	54 ± 5	1.9 ± 0.07	6.71 ± 0.08	0.71 ± 0.03	1.385 ± 0.005	1.9 ± 0.07	6.71 ± 0.08	0.71 ± 0.03	1.385 ± 0.005							
NC <sub>L</sub> -CLV/10	47	103.3 ± 0.5	1.765 ± 0.006	586.4 ± 0.9	0.49 ± 0.08	3008 ± 62	67.0 ± 0.5	60 ± 3	36.5 ± 0.5	2133 ± 59	47.1 ± 0.2	1.6 ± 0.2	6.1 ± 0.4	1.67 ± 0.08	1.074 ± 0.005	1.6 ± 0.2	6.1 ± 0.4	1.67 ± 0.08	1.074 ± 0.005							
NC <sub>L</sub> -SSL/5	12	86.7 ± 0.4	2.128 ± 0.002	8.0 ± 0.4	0.53 ± 0.06	3317 ± 184	55 ± 11	55 ± 29	20.7 ± 0.6	1222 ± 28	40 ± 8	2.3 ± 0.2	4.0 ± 0.2	1.75 ± 0.03	1.551 ± 0.006	2.3 ± 0.2	4.0 ± 0.2	1.75 ± 0.03	1.551 ± 0.006							
NC <sub>L</sub> -SSL/5	29	126.9 ± 0.6	1.148 ± 0.010	12.0 ± 0.3	1.40 ± 0.08	4045 ± 33	102 ± 10	1410 ± 212	43.2 ± 0.5	1348 ± 45	79 ± 11	1.98 ± 0.06	5.5 ± 0.2	1.70 ± 0.03	1.599 ± 0.006	1.98 ± 0.06	5.5 ± 0.2	1.70 ± 0.03	1.599 ± 0.006							
NC <sub>L</sub> -SSL/5	47	134.1 ± 0.6	0.972 ± 0.002	18.2 ± 0.2	1.46 ± 0.02	3935 ± 34	74 ± 14	975 ± 85	31 ± 5	1087 ± 42	34.8 ± 1.3	1.27 ± 0.07	8.3 ± 0.2	1.31 ± 0.03	1.809 ± 0.004	1.27 ± 0.07	8.3 ± 0.2	1.31 ± 0.03	1.809 ± 0.004							
NC <sub>L</sub> -SSL/10	12	157.0 ± 0.8	1.430 ± 0.008	11.36 ± 0.13	1.232 ± 0.11	3210 ± 73	126.5 ± 1.1	1884 ± 23	109 ± 23	983 ± 30	118 ± 3	23.5 ± 0.2	5.8 ± 0.3	2.97 ± 0.03	5.83 ± 0.02	23.5 ± 0.2	5.8 ± 0.3	2.97 ± 0.03	5.83 ± 0.02							
NC <sub>L</sub> -SSL/10	29	173.68 ± 0.06	0.530 ± 0.002	8.47 ± 0.06	1.230 ± 0.09	3924 ± 24	71 ± 3	1419 ± 23	93 ± 4	528 ± 12	29.9 ± 0.8	14.4 ± 0.2	4.41 ± 0.02	1.03 ± 0.03	4.946 ± 0.009	14.4 ± 0.2	4.41 ± 0.02	1.03 ± 0.03	4.946 ± 0.009							
NC <sub>L</sub> -SSL/10	47	168.7 ± 0.8	0.572 ± 0.003	22.12 ± 0.09	1.466 ± 0.12	3270 ± 31	68.8 ± 1.4	1403 ± 20	55.7 ± 0.3	458 ± 59	24.0 ± 0.4	10.86 ± 0.07	14.1 ± 0.2	1.47 ± 0.03	5.381 ± 0.009	10.86 ± 0.07	14.1 ± 0.2	1.47 ± 0.03	5.381 ± 0.009							

### 3.2. Addition of organic amendments. Principal component and hierarchical clustering analyses

We employed a Principal Component analysis to identify the key parameters describing data variability from the results. We first performed a validation of variables, observing communalities, the ratio number of variables/cases, possible missing data and comparing the results of the standardized/non-standardized matrix. Subsequently, fourteen analytical variables were selected: Cl<sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, acetate, formate, oxalate, phthalate. A PCA was then initiated with the variables selected plus the dose (*D*) of the amendments and the incubation time (*t*), in order to determine the influence of time and dose on soil treatments. The results (Table 4) indicated that the incubation time was related only to the formate ion whilst the dose was clearly associated with ions provided by SSL in PC1 and with NO<sub>3</sub><sup>-</sup> provided by CLV in PC3. This way, only dose has an influence on soil solution composition after organic amendments, and consequently constitutes a variable to be kept in mind in the selection of the final amendment. Thus, the PCA extracted four Principal Components (PCs) which explain a total variance of 88%. The first PC (variance of 47%) included the variables phthalate, K<sup>+</sup> and NH<sub>4</sub><sup>+</sup> followed by Cl<sup>-</sup>, acetate, PO<sub>4</sub><sup>3-</sup> and Na<sup>+</sup>, Mg<sup>2+</sup> and oxalate were included in PC1 with minor loadings. The cases associated with PC1 are SSL-amended soils, mainly at the 10% dose, and with scores decreasing slightly from 12 to 47 days.

PC2 (variance of 20%) was formed by NO<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and Ca<sup>2+</sup> and represents the ions whose levels increased by liming. The limed soil most representative of this PC was the one incubated up to day 47 (NC<sub>L47</sub>), indicating that liming effects are long lasting in time.

PC3 (variance of 11%) explains the high content of NO<sub>3</sub><sup>-</sup> of the composted sewage sludge, mainly at the 10% dose. In this PC the most representative amended soils were CLV<sub>1047</sub> > CLV<sub>1029</sub> > CLV<sub>1012</sub>, indicating that incubation with CLV<sub>10</sub>-amendment increased NO<sub>3</sub><sup>-</sup> in soil.

Finally, PC4 (variance of 9%) explains the relationship between time (*t*) and formate. The most representative samples were the soils corresponding to day 47, indicating the influence of incubating time for formate.

Fig. 2 shows how PCA groups differentiate soil types. PC1 separates the native soil from all amended soils -including limed soil-; thus, the amended and limed soils have a higher content of ions, representative of PC1 and PC2. Moreover, PC2 discriminates soil with stabilized sewage sludge (SSL<sub>10</sub> > SSL<sub>5</sub>) from limed soil and composted sewage sludge (CLV and NC<sub>L</sub>), but fails to discriminate completely between the doses of CLV. Besides, it is confirmed that incubation time is in no case related with ion release. Thus, SSL application would provide nutrients to soils and plants and would constitute a strategy for restoring this mining area through the development of a vegetative cover (Mingorance et al., 2014).

Samples were classified by cluster analysis, which revealed two main clusters of samples at >75% relative Euclidean linkage distance, one of them made up of SSL<sub>10</sub> soils (*C1*) and the other of the remaining ones (*C2*) (Fig. 3) confirming the high grade of discrimination of SSL<sub>10</sub> amendment. Comparing the different days of incubation, the variable time has again no influence on the results and does not affect ionic soil solution composition. Within *C2*, cluster *C2b* corresponds to the other well-discriminated samples, the native soils. However, complete separation occurs at around 17% relative Dlink, where cluster *C2a* comprises four sub-clusters: *C2a1* and *C2a2*, which represent a low level of discrimination between SSL<sub>5</sub> and CLV<sub>10</sub> soils, and *C2a3* and *C2a4* between CLV<sub>5</sub> and NC<sub>L</sub> soils. Additionally, the clustering tree indicates a trend of increasing nutrient levels from NC<sub>L</sub> (upper side) to SSL<sub>10</sub> (lower side) soils.

**Table 4**

Result of the PCA from matrix comprising 16 variables (cations, anions, dose and time) and 18 cases.

PC	Eigenvalue	Variance (%) / Cum. var. (%)	Sign	Variables	Cases	Source/Interpretation
PC1	8.06	47.4/47.4	+	Phtalate (0.95), K <sup>+</sup> (0.94), NH <sub>4</sub> <sup>+</sup> (0.94), D <sub>SSL</sub> (0.92), Cl <sup>-</sup> (0.91), acetate (0.88), PO <sub>4</sub> <sup>3-</sup> (0.85), Na <sup>+</sup> (0.85) > Mg <sup>2+</sup> (0.72), oxalate (0.72)	+ SSL10 <sub>12</sub> >> SSL10 <sub>29</sub> , SSL10 <sub>47</sub> >> SSL5 <sub>29</sub> , SSL5 <sub>47</sub>	Components that increase with SSL-amendment and dose (D)
PC2	3.43	20.2/67.6	+	NO <sub>2</sub> <sup>-</sup> (0.88), SO <sub>4</sub> <sup>2-</sup> (0.85), Ca <sup>2+</sup> (0.82)	+ NC <sub>L47</sub> >> NC <sub>L12</sub> , NC <sub>L29</sub> , CLV5 <sub>29</sub>	Components that increase with liming
PC3	1.93	11.3/78.9	+	NO <sub>3</sub> <sup>-</sup> (0.96), D <sub>CLV</sub> (0.95)	+ CLV10 <sub>47</sub> , CLV10 <sub>29</sub> , CLV10 <sub>12</sub> >> CLV5 <sub>12</sub> , CLV5 <sub>47</sub>	Increase of nitrate with CLV amendment and dose (D)
PC4	1.55	9.2/88.1	+	t (0.92) >> formate (0.60)	+ SSL10 <sub>47</sub> > SSL5 <sub>47</sub> , CLV5 <sub>47</sub>	Increase of formate with time (t)

Loadings are in parentheses.

### 3.3. Addition of organic amendments. Inorganic ions in the soil solution

On the first collection date, cation content tended to increase with amendment dose, with the exception of Ca<sup>2+</sup>, which was very abundant as a result of liming (Table 3). This increase was only significant for NH<sub>4</sub><sup>+</sup> in CLV ( $r = 0.999$ ,  $p = 0.035$ ). The effect of the dose was less relevant at the end of incubation ( $t = 47$  d) and affected K<sup>+</sup> after SSL addition ( $r = 1.000$ ,  $p = 0.003$ ) and NH<sub>4</sub><sup>+</sup> in CLV- amended soil, although not significantly ( $p = 0.174$ ).

Incubation time led to a decrease in the soil solution concentration of all cations (Fig. 4) in the SSL-amended soil, -but not in the CLVs-, due to cation-induced coagulation, which preferentially removes functional groups from the humic acids involved in metal-cation binding in the soil solution (Christl and Kretzschmar, 2007). Recently Cornu et al. (2011) suggested that during soil incubation, the composition of DOM may shift to more aromatic compounds, assimilated to humic substances, with a higher cation affinity. The decrease for SSL<sub>10</sub> was greater than for SSL<sub>5</sub> and more pronounced for the bivalent cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>), with a higher charge density ( $\rho_+ = Q_+/V_+$ ), than for the monovalent ones (Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>) (Fig. 2). The decline in concentration was fitted for the different cations to a first order exponential equation with  $R^2$  ranging from 0.76 for NH<sub>4</sub><sup>+</sup> to 0.93 for K<sup>+</sup>. The time required for the disappearance of half of the initial ion concentration ( $t_{1/2\downarrow}$ ) for SSL<sub>10</sub> was significantly related for each cation with the corresponding charge density according to the equation  $t_{1/2\downarrow} = 41.86 - 0.208\rho_+$ ,  $R^2 = 0.918$ , excepting for NH<sub>4</sub><sup>+</sup>, whose slow disappearance (Wu et al., 2014) from the soil solution involves additional mechanisms other than electrostatic interactions, such as oxidation processes in N species -nitrification to nitrite and then to nitrate-.

As for anions, we found no relationship with amendment dose for CLV ( $p > 0.05$ ), whilst when NC<sub>L</sub> was amended with SSL, Cl<sup>-</sup> in soil solution increased proportionally to the dose ( $r = 1.000$ ,  $p = 0.000$ ) and SO<sub>4</sub><sup>2-</sup> decreased, almost reaching statistical significance ( $r = 0.996$ ,  $p = 0.055$ ). Phosphate nutrient was also provided by SSL, increasing exponentially with the dose ( $R^2 = 0.80$ ). NC soil contains large amounts of Al and Fe hydrous oxides and can therefore adsorb large amounts of added P. SSL amendment may compete with phosphate for specific adsorption sites on soil Fe/Al oxides (Cui et al., 2011), thus releasing some soil-adsorbed P into the soil solution and increasing soil available P (Wu et al., 2014). The increase in P throughout incubation in SSL (Fig. 5) is therefore due to a combination of increased pH and decreased exchangeable Al by liming, and the amount of mineralized P provided by the amendments (Guppy et al., 2005; Iyamuremye et al., 1996). Anion release to the soil solution was not significantly related with incubation time for any amendment ( $p > 0.05$ ).

The behaviour of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> merits particular attention and may be explained by the red-ox reactions involved in the above mentioned NH<sub>4</sub><sup>+</sup> oxidation processes and attributed to the high contents of NO<sub>3</sub><sup>-</sup>-NH<sub>4</sub><sup>+</sup> in CLV and SSL pure amendments (Table 5). In SSL-amended soil a 3-fold decrease was observed for nitrite whilst, at the same time, ammonium was seen to disappear (SSL<sub>10</sub>) and nitrate was formed, confirming the aforementioned reactions (Figs. 5 and 6). On the other hand, when NC<sub>L</sub> was amended with CLV, nitrite levels increased, while those of ammonium showed a decrease and nitrate remained stable (Table 3). Consequently, when the pure amendment contains a high content of ammonium but not of nitrate (SSL), nitrification reactions



proceed until the final nitrate product (Wu et al., 2014), but when the pure amendment contains a high content of nitrate but not of ammonium (CLV), nitrification reactions cease at the intermediate nitrite product.

If all the ions in the soil solution were to be uptaken by plants, problems would arise with  $\text{SO}_4^{2-}$ , present in native mine soil and enhanced by liming with high levels, but especially with  $\text{NH}_4^+$ , which largely exceeds the normal values, due to addition of organic amendments (SSL >>> CLV, 10% > 5%) (Table 5). Finally,  $\text{NO}_3^-$  could constitute a hazard when amending with CLV at 10%. A dose <10% would therefore be better suited for plant establishment, in agreement with previous studies involving the growth of tomato (*Lycopersicon esculentum* Mill.), rye grass (*Lolium perenne* L.) and ahipa (*Pachyrhizus ahipa* (Wedd.) Parodi) in  $\text{N}_{\text{CL}}$  soil amended with different SSL doses (Mingorance et al., 2014).

**Table 5**

Organic and inorganic ion content of amendments (mean  $\pm$  standard deviation,  $n \geq 4$ ): stabilized (SSL) and composted (CLV) sewage sludge and Carbocal (CAL).

Ion	CLV (mg kg <sup>-1</sup> )	SSL (mg kg <sup>-1</sup> )	CAL (mg kg <sup>-1</sup> )
Cl <sup>-</sup>	825 $\pm$ 195	2137 $\pm$ 149	18.2 $\pm$ 0.5
NO <sub>2</sub> <sup>-</sup>	3.68 $\pm$ 0.08	3.93 $\pm$ 0.14	1.4 $\pm$ 0.2
NO <sub>3</sub> <sup>-</sup>	14,922 $\pm$ 1718	61 $\pm$ 4	14.9 $\pm$ 0.5
PO <sub>4</sub> <sup>3-</sup>	8.2 $\pm$ 0.3	156 $\pm$ 13	4.9 $\pm$ 0.7
SO <sub>4</sub> <sup>2-</sup>	3811 $\pm$ 298	3300 $\pm$ 305	3085 $\pm$ 133
Na <sup>+</sup>	653 $\pm$ 118	434 $\pm$ 28	22 $\pm$ 4
NH <sub>4</sub> <sup>+</sup>	630 $\pm$ 84	4966 $\pm$ 307	2.6 $\pm$ 0.6
K <sup>+</sup>	1187 $\pm$ 100	1362 $\pm$ 72	4.8 $\pm$ 0.6
Ca <sup>2+</sup>	473 $\pm$ 90	273 $\pm$ 19	4303 $\pm$ 133
Mg <sup>2+</sup>	63 $\pm$ 4	105 $\pm$ 19	176 $\pm$ 19
C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	4.0 $\pm$ 0.3	35 $\pm$ 7	0.61 $\pm$ 0.04
CHO <sub>2</sub> <sup>-</sup>	68 $\pm$ 5	58 $\pm$ 6	3.03 $\pm$ 0.11
C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	2.2 $\pm$ 0.3	10.1 $\pm$ 1.8	8.8 $\pm$ 0.3
C <sub>8</sub> H <sub>4</sub> O <sub>4</sub> <sup>2-</sup>	14 $\pm$ 3	37 $\pm$ 6	9.8 $\pm$ 0.6

#### 3.4 Addition of organic amendments. Organic anions in the soil solution

In all cases, amendment addition increased the total content of LMWOAs when compared to limed soil ( $p > 0.05$ ) (Table 3). Addition of SSL caused a higher total load of LMWOAs ( $p < 0.001$ ) than CLV ( $p < 0.006$ ) and proportionally to the dose, in agreement with previous data (Hees et al., 2000; Strobel, 2001). SSL-amendment exhibits higher values of oxalate, phthalate and mainly acetate than CLV-amendment (Table 5). Acetate and formate were the most abundant organic acids: Following the big decrease caused by liming, formate increased with both amendments and doses (14-17-fold for 10% dose,  $p < 0.05$ ), while acetate increased only for SSL (11-fold for 10%,  $p = 0.003$ ); however the increase in oxalate (only 2-fold,  $p = 0.108$ ) and in phthalate ions (12-fold for 10%,  $p = 0.0002$  and 4-fold for 5%) is only slightly appreciable for SSL-amended soil, as already seen in PC1 and also in the composition of both pure amendments.

Although LMWOAs are present in the soil solution, they are also adsorbed on the soil surface. According to Strobel (2001) aliphatic acids are more hydrophilic and soluble in soil solution than aromatic acids and therefore a larger fraction of the former is usually encountered in solution. This agrees with the lower concentration of phthalate found in comparison with aliphatic acids, mainly in CLV. Additionally, although other anions were not determined in this investigation, a larger fraction of di- or tri-carboxylic acids is expected to be sorbed compared with monocarboxylic acids, in line with the higher concentrations of acetate and formate found with both amendments.

With regard to the incubation process, results showed that total LMWOA content tended to decrease in SSL-amended soils (Table 3). Decreases were significant for oxalic and acetic acids ( $p = 0.010$ ) in SSL<sub>5</sub> soils ( $t_{1/2\downarrow}$  was 42 days,  $R^2 = 0.96$  for acetate and 74 days,  $R^2 = 0.90$  for oxalate) and proportional to the dose in SSL<sub>10</sub> ( $t_{1/2\downarrow}$  was 32 days,  $R^2 = 0.97$  for acetate and 36 days,  $R^2 = 0.87$  for oxalate). Moreover, formate increased during incubation at both doses ( $t_{1/2\uparrow}$  was 19 days,  $R^2 = 0.98$  for SSL<sub>5</sub> and 16 days,  $R^2 = 0.55$  for SSL<sub>10</sub>), in accordance with the negative correlation between oxalate and formate also identified in the 2.1 section. This repetitive behaviour further supports the hypothesis that formate constitutes a secondary decomposition product of other organic acids from soils. Küsel et al. (1999) reported that the aliphatic LMWOAs (acetate, formate and oxalate) constituted a substrate for a greater number of microorganisms than aromatic acids. This explains the decrease in acetate with incubation time and the increase in oxalate and formate, which are the final products in the decomposition pathway of more complex organic acids in soil with both kinds of amendments.

Additionally, the decline with incubation time of some organic acids, which are present at a higher concentration in 10% SSL-amended soil, coincides with the decline observed for cations. This decline may be

due to the formation of some cation-LMWOA complexes (Ali and Dzombak, 1996a,b) and to immobilisation through cation-binding to LMWOAs sorbed on the soil surface. These complexes are especially described for di- and tri-carboxylic acids such as phthalate, although the same behaviour was observed in our case for oxalate. Since several organic acids have yet to be actually determined, our future research entails evaluating a compendium of other additional organic acids, mono and poly-carboxylic, such as citrate, malate, tartrate, succinate, lactate, as well as aliphatic and aromatic organic acids, in other incubation experiments.

Soil fertility is related to nutrient concentration in the soil solution, from which plants obtain mineral nutrients through root uptake. Therefore, high nutrient concentrations in the soil solutions should be available during the plant growing stage. Addition of organic amendment favours uptake by plants of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and formate in CLV, and  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ , acetate, phthalate and formate in SSL, and the retention-sorption by soils of  $\text{NO}_3^-$  in CLV, and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{SO}_4^{2-}$  in SSL.

#### 4. Conclusions

Organic amendments from fresh or composted sewage sludge induced a drastic change in soil solution composition with a possible impact for future plant development. The multivariate analysis showed that soil solution content is mainly affected by the addition of ions from the corresponding organic amendments, but we observed a dose-dependent effect only for SSL. Nitrite may accumulate in the soil solution after addition of CLV, as a result of nitrification reactions and consequently, levels of nitrite, nitrate and ammonium will depend upon the composition of the pure amendments. Furthermore, the amount of LMWOAs tended to increase with the dose of organic wastes (SSL and CLV), with higher levels of acetate and formate, aliphatic organic acids of short chain length. With respect to incubation time, results show that the LMWOAs content tended to present a general decrease in SSL-amended soil, mainly due to the decrease in oxalate and acetate. Additionally, soil incubation resulted in a decrease in soluble cations, particularly in SSL-amended soil, a fact that could be linked with the overall decrease in LMWOAs. Finally, organic acids, which because of their lability can be utilized by most soil microorganisms and which play a key role in the mobilization of nutrients from the rhizosphere, represent only between 0.13 and 0.58% of the total soil solution, even in soil amended with organic residues.

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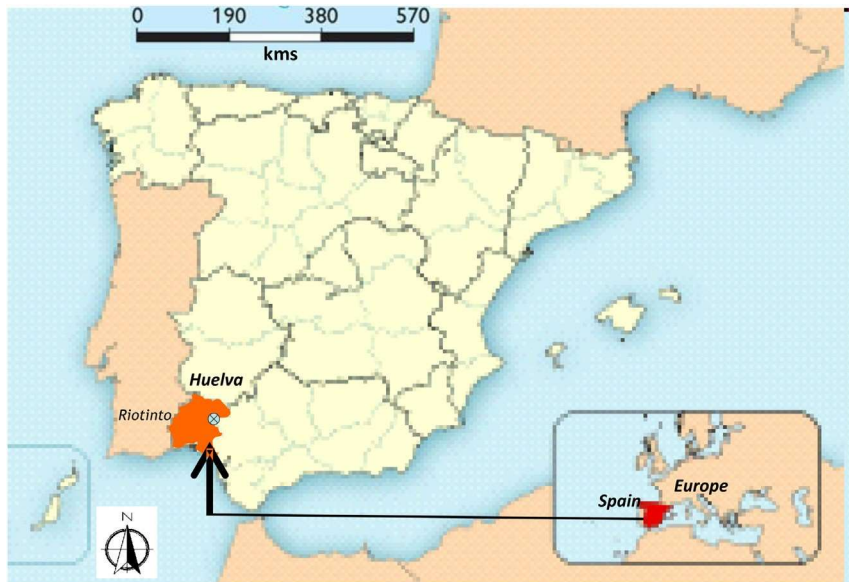


Fig. 1. Location of the Nerva mine in the Riotinto area, Huelva (SW Spain, UTM: 29S 715131 4175471).

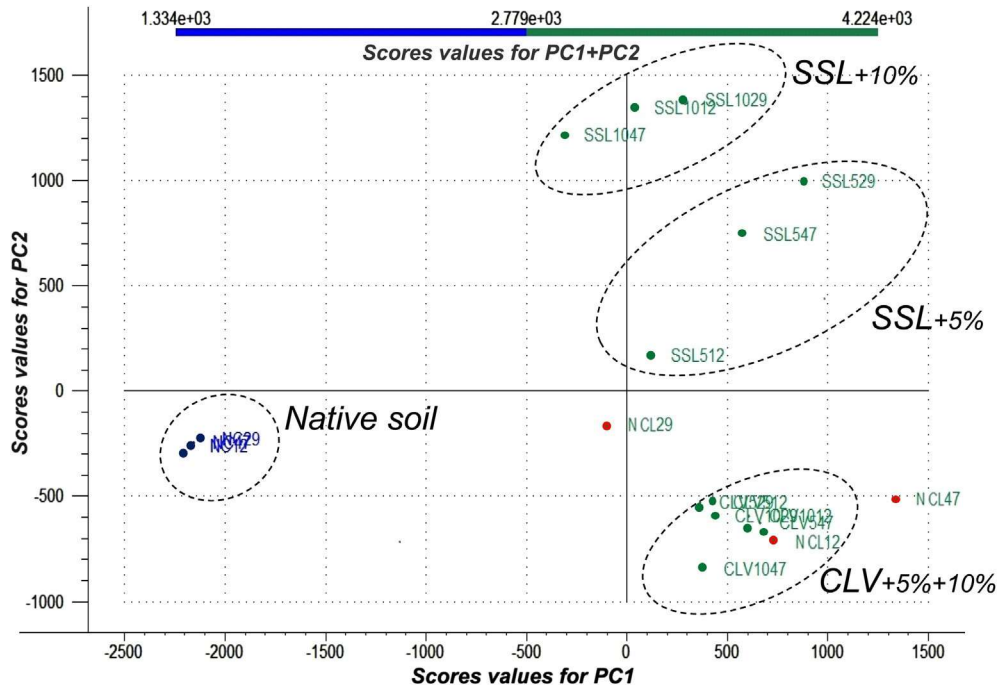


Fig. 2. Map of scores for PC1 and PC2 for all samples. Discrimination of groups of soils.

Dendrogram of samples, Ward's method

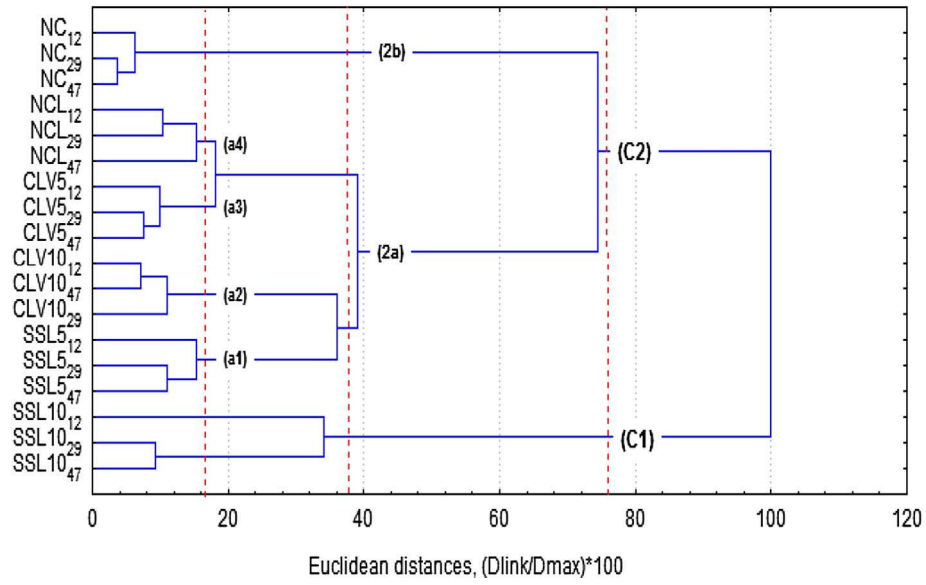
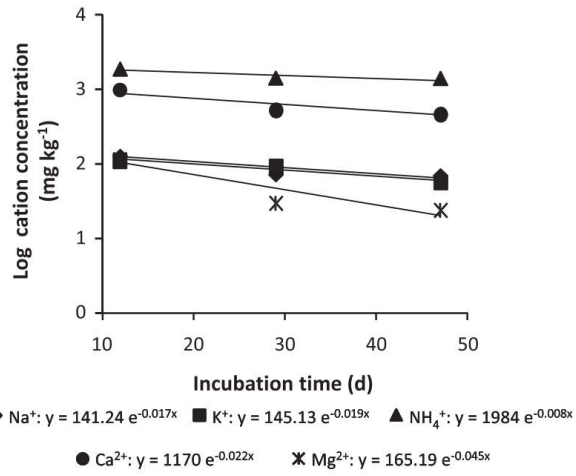


Fig. 3. Hierarchical clustering results (dendrogram) of ion concentrations for Native, NC, CLV and SSL-amended soils.



**Fig. 4.** Decrease in cation content with incubation time in days (d) in stabilized sludge-amended soil at 10% (SSL<sub>10</sub>). Fitting equations for each cation are also presented.



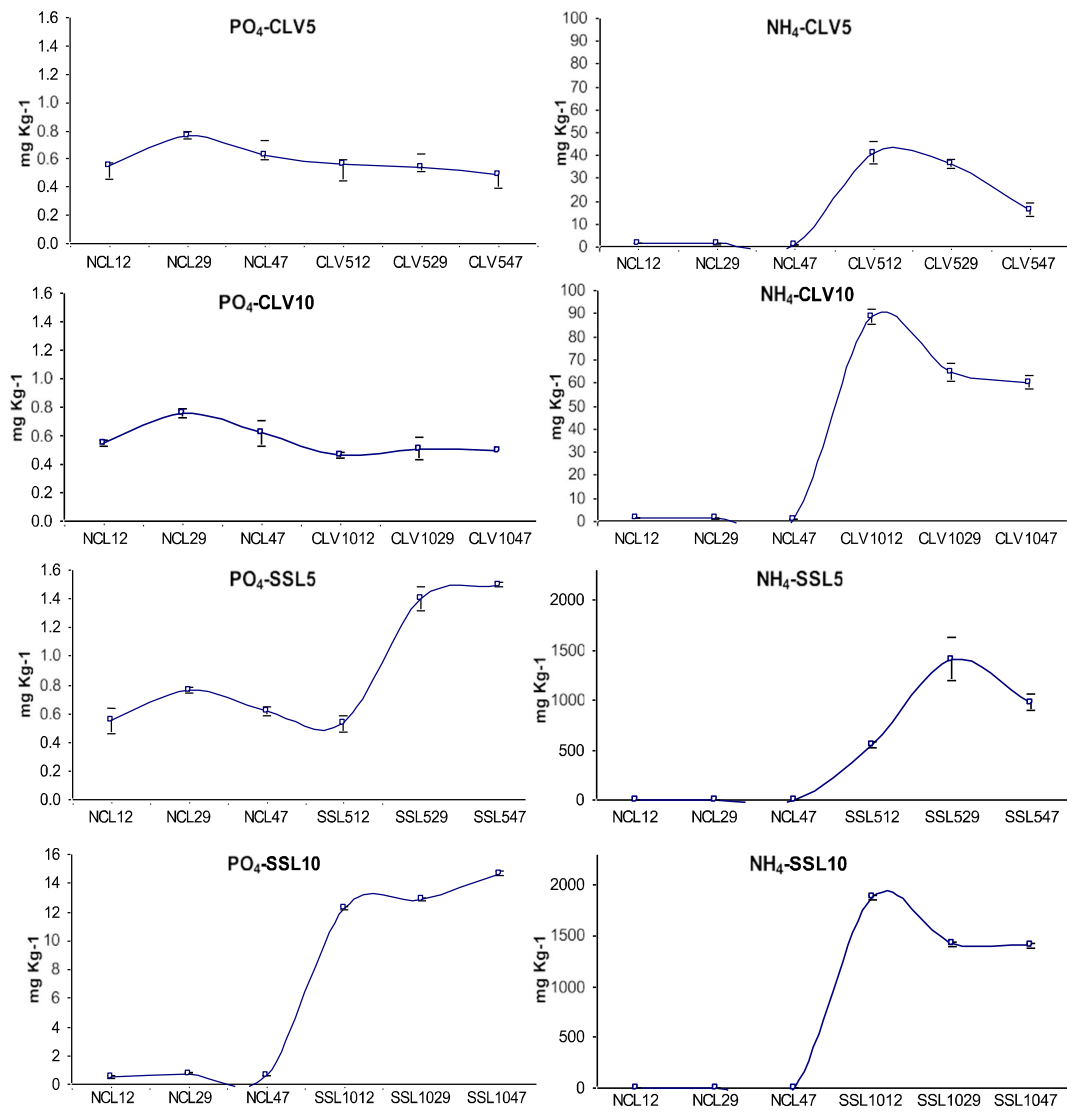


Fig. 5. Distribution of soil solution concentrations for the nutrients PO<sub>4</sub><sup>3-</sup> and NH<sub>4</sub><sup>p</sup> along the different amendments and incubation time.

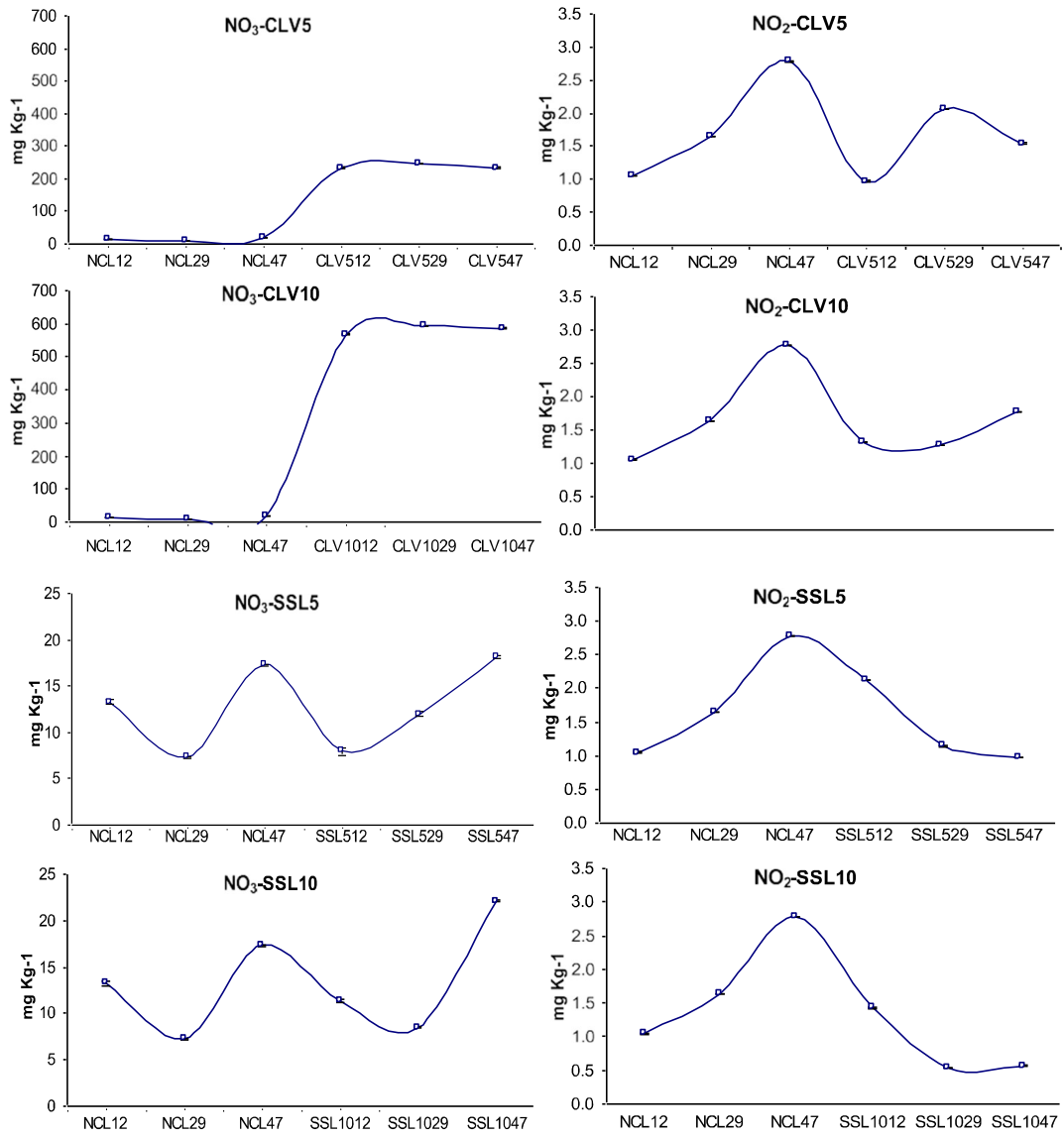


Fig. 6. Distribution of soil solution concentrations for the nutrients NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> along the different amendments and incubation time.

