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Oxide weathering and trace metal release by bacterial reduction in a New Caledonia Ferralsol

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Abstract. Bacterial reduction of Fe- and Mn-oxides was studied in a surface horizon of a New-Caledonian Ferralsol in batch experiments. Two treatments were imposed containing different sources of organic matter (soil organic matter with or without glucose addition) to link organic matter biodegradation with reduction process. The concomitant solubilization of Ni and Co was also studied. Results showed that anaerobic Fe- and Mn-reducing bacterial activity was responsible for Fe- and Mn-oxide solubilization by anaerobic respiration or fermentation. When C was more available, oxide reduction was enhanced. Mn-oxide appeared as the major reducible phase and metal source rather than goethite. Co and Ni were solubilized with Fe and Mn but their amounts in solution decreased at the end of experiment. The bioavailability of heavy metals in this soil was increased by biological reduction but was limited by adsorption or precipitation phenomena.

Introduction

In New Caledonia, a large part (one third) of the soils is developed on ultramafic rocks. Ferralsols derived from these rocks are dominated by iron (Fe) and manganese (Mn) oxides (Nalovic & Quantin 1972; Schwertmann & Latham 1986; Quantin et al. 1997). These soils contain very high amounts of nickel (Ni) and other transition metals such as cobalt (Co) and chromium (Cr). Fe- and Mn-oxides are major sinks for metals (McKenzie 1989; Singh & Gilkes 1992; Trolard et al. 1995) and play an important role in controlling their availability (Francis & Dodge 1990; Gasser et al. 1996; Bousserrhine et al. 1999).

The presence of some Ni hyperaccumulator plants (Jaffré et al. 1976), the high contents of Ni in crops (L'Huillier & Edighoffer 1996), and the

agronomic difficulties in crop production suggest heavy metal toxicity for cultivated plants.

Understanding metal transfer to the soil solution and to the plants is of considerable interest for environmental and soil sciences (Adriano 1986). Previous results have shown a higher Ni availability in soil series of southern New Caledonia which appears to be highest in colluvio-alluvial and plain soils subjected to temporary water tables (waterlogging and probably reducing conditions) (Becquer et al. 1995; L'Huillier & Edighoffer 1996). Ni is also observed in the soil solution collected from these fields (Becquer et al. 1997). Reduction processes may increase the metal availability with the possible occurrence of mangani- and ferri-reducing microorganisms (Berthelin 1982, 1988; Francis & Dodge 1988, 1990; Bousserrhine et al. 1999; Markwiese & Colberg 2000) as well as chemical processes.

In fact, reduction processes may involve facultative anaerobic or strict anaerobic bacteria which use organic matter as carbon and energy sources and as electron donors for their growth and their reducing activity (Lovley & Phillips 1988; Lovley 1991; Nealson & Myers 1992; Ehrlich 1996; Bousserrhine et al. 1999; Madigan et al. 2000). An increase in anaerobic bacterial activity due to biodegradation of organic matter may have an appreciable effect on the dissolution of oxides and associated metals.

The aim of the present paper was to determine the possible role of Mn- and Fe-reducing bacteria in heavy metal (mainly Ni and Co) solubilization in relation to organic matter biodegradation and to identify the sources of solubilized heavy metals.

Material and methods

Soil sampling and analysis

Soil samples were collected in southern New Caledonia in the Ouénarou forestry station. It originated from the lower part of a soil sequence previously studied by Becquer et al. (1995). The soil is formed from colluvial-alluvio materials and subjected to temporary waterlogging as shown by the presence of black nodules of Mn oxide in the soil profile. It exhibits high contents of exchangeable and extractable Ni (Becquer et al. 1995). Soil was collected from the sub-surface horizon (4–10 cm) and sieved at 2 mm. One part was air dried for analysis, while the remaining soil was kept moist (in the dark at 4 °C) until the experiment was initiated.

Total C and N in the solid phase were quantified using a CHN 1108 Carlo Erba analyser. The pH was measured in water suspension using 1:2.5 (w/w) soil:water ratio.

Fe and Mn concentrations in the sample were measured by ICP-AES (Jobin-Yvon 238) after dissolution in lithium metaborate at 1000 °C. Ni and Co contents were determined by ICP-AES after diacid digestion (2:1 HNO_3:HCl ratio). Reductive dissolution of Fe-oxides was studied using a modified DCB method after Holmgren (1967) (75 mg soil, 250 mg dithionite and 25 ml citrate-bicarbonate solution, 25 °C for 5 days). The hydroxylamine hydrochloride procedure of Chao (1972) was used for Mn-oxides dissolution. Metal concentrations were measured as described above.

Experimental design

Batch incubations were performed with 50 g (or 25 g for controls) soil in hermetically sealed 1000-mL (or 500-mL) plasma bottles, supplemented with 750 mL (or 375 mL) liquid medium. All incubations were carried out anaerobically, under O_2-free N_2 atmosphere (suspensions were flushed with N_2 for 15 mn) with four replicates per treatment.

Two types of treatments were done with different sources of organic matter. One, with autochthonous soil organic matter as the only source of carbon (samples incubated with distilled water as liquid medium; SOM treatment). The other treatment was supplied with a glucose solution (1 $g.L^{-1}$ or 6 g $C.kg^{-1}$ of soil; GLU treatment). Glucose was used as a simple compound originated from cellulose decomposition. Flasks with soil were incubated in the presence or absence of microorganisms, i.e. under biotic or abiotic conditions, to distinguish microbial and physico-chemical processes. Abiotic conditions were obtained by addition of 1 $g.L^{-1}$ of Na-thimerosal.

The batch cultures were incubated in the dark at 28 °C for 11 weeks, without shaking except hand shaking prior gas sampling.

Analytical procedures

Bacterial activity was first quantified by measuring the mineralization of organic matter. Periodically, a 4-mL portion of the flask headspace was sampled with a syringe and analyzed for its CO_2 content with a Binos 1004 infrared spectrophotometer. Biodegradation and fermentation activities were followed by measurement of dissolved organic carbon (DOC) using a total carbon analyser (Dohrmann DC190) and by organic acids production in the supernatant. They were identified by high performance liquid chromatography (HPLC) (Gold Beckman system) equipped with a UV detector set at 200 nm (Aminex HPx87H anion exchange column under isocratic conditions).

Fe, Mn and heavy metals contents in solution (Fe_d and Mn_d) were determined at various times by collecting 15 mL (or 10 mL for controls) of

suspension with a syringe under sterile conditions. The changes in volume were considered in all calculations. The suspensions were filtered through 0.2 μm pore-size membranes (Sartorius). Aliquots of supernatants were analyzed for total metal concentration by ICP-AES (Jobin Yvon 238). pH and Eh of the medium were also measured. The soluble Fe^{2+} concentration was determined colorimetrically using 1 mL filtered solution and 1 mL 0.5% orthophenantroline solution adjusted to 25 mL. The absorbance was measured after 25 mn at 490 nm (Beckman DU-70 Spectrophotometer). To better estimate the iron reduction, a simple 0.5 N HCl extraction (Lovley & Phillips 1986) was done to solubilized the portion of Fe(II) that is present in the solid phase either sorbed or precipitated (Tugel et al. 1986). The Mn^{2+} concentration was measured colorimetrically using the red-brown complex of manganese with formaldoxime. 100 μL formaldoxime solution (8 g hydroxylamine hydrochloride and 4 mL 36% w/w formaldehyde in 200 mL of distilled water) and 100 μL 5–6 M ammonia solution were added to 2 mL of filtered solution. After 2 mn, 100 μL 0.1 M EDTA solution and 200 μL 10% hydroxylamine hydrochloride solution were added. The absorbance was measured at 450 nm, 10–15 mn later. The difference between total Fe or Mn and Fe^{2+} or Mn^{2+} was considered as metal complexed by organic matter.

Statistical analysis

Correlation coefficients (r) were calculated between all measured variables and tested using Ficher transformation. Significant correlation were retained for $p \leq 0.01$ or $p \leq 0.001$. All calculations were done using Statview Software version 4.02 (Abacus Concepts, Inc.).

Results

Soil characteristics

The soil had a high content of iron and relatively high contents of manganese, nickel and cobalt (Table 1). The organic matter of soil sample reached 5% and the C:N ratio was 22.

More than 95% of total iron was dissolved by DCB reagent. EDS analysis and MET observations showed that Fe was mainly in well-crystallized, needle-shaped goethite (results not shown). DRX showed the presence of hematite traces. Moreover, DCB also extracted more than 95% of total Ni. Hydroxylamine hydrochloride solubilized 50% of total Mn and Co, and only 1% of total Ni. These results suggested that Mn and Co were mainly associated in Mn-oxides and Ni in Fe-oxides. Further details of the chemical and

Table 1. Main characteristics of Ouénarou soil sample (total content of Fe, Mn, Ni and Co).

Depth	pH_{H_2O}	Corg	C/N	Fe	Mn	Ni	Co
(cm)		(%)		$g.kg^{-1}$		$mg.kg^{-1}$	
4–10	4.6	2.60	21.7	397	4.68	7750	720

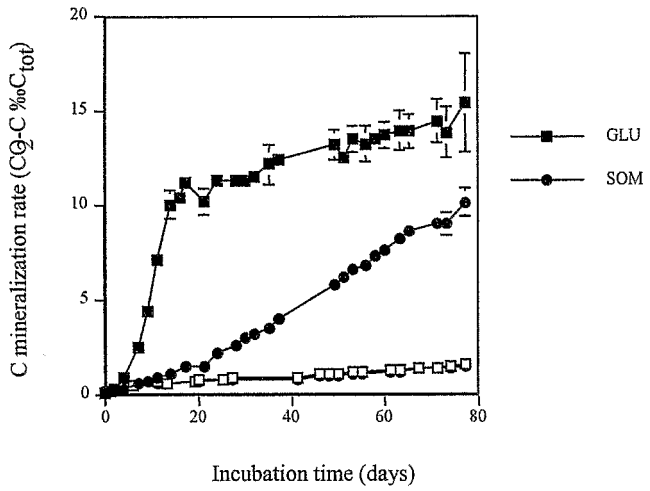

Figure 1. C mineralization in experiments with (GLU) and without (SOM) glucose addition, under biotic (filled symbols) and abiotic (open symbols) conditions.

mineralogical characteristics of the soil are given by Becquer et al. (1995) and Quantin et al. (1997).

Bacterial activity

During the batch incubation, CO_2 production in controls was negligible and attributed only to chemical phenomena (Figure 1). In contrast, CO_2 from organic matter mineralization in biotic treatments corresponded to 15.3 and 10.1‰ of the total C content for the GLU and SOM treatments, respectively after 11 weeks of incubation (Figure 1). Microscopic observations and plate counting of microorganisms showed that only bacteria ($3.5\ 10^5$ $CFU.g^{-1}$ soil) were growing in these experimental conditions.

In the treatment where bacterial activity was stimulated by glucose addition (GLU), after a lag-period of 2 days, the CO_2 increased strongly with time until 17th day with an average mineralisation rate of 0.714‰ $C_{tot}.d^{-1}$. After this period, it reached a slower mineralization rate of 0.073‰ $C_{tot}.d^{-1}$.

DOC concentrations stayed high and relatively constant ca. 300 $mg.L^{-1}$ with some non significant variations with time. HPLC analysis revealed the presence of fermentation products, in particular acetic, propionic and butyric acids. Acetic and propionic acids concentrations reached 9.68 $mmol.L^{-1}$ and 0.947 $mmol.L^{-1}$, respectively. Butyric acid remained close to 0.9–1.0 $mmol.L^{-1}$. Glucose was not detected in supernatants by HPLC after few days of incubation.

In the SOM treatment, the mineralization rate of the natural organic matter was low (0.075‰ $C_{tot}.d^{-1}$ in average) for the first 21 days. After this period, the mineralization rate increased to 0.151‰ $C_{tot}.d^{-1}$. DOC remained at a low level (ca. 20 $mg.L^{-1}$) during the duration of the experiment. No organic acids were detected in the incubation solution by HPLC analysis.

Eh, pH, Mn and Fe evolution

Abiotic treatments

In the abiotic controls without bacterial activity, no changes in Eh or Fe content were observed. Eh was constant around 380 mV. Dissolved Fe (Fe_d) and Mn (Mn_d) concentrations were around 4 $mg.L^{-1}$ and between 10 to 20 $mg.L^{-1}$, respectively. No Fe^{2+} solubilization occurred; however, Mn^{2+} was measurable in both (GLU and SOM) abiotic treatments (Figures 2(b) and 4(b)). pH increased from 5.5 to 6.2 during the first 3 weeks and remained constant after that (Figure 3). No differences occurred between GLU and SOM abiotic treatments for all these parameters.

Biotic incubation with glucose addition (GLU)

For GLU treatment under biotic conditions, Eh decreased rapidly from 326 mV to 150 mV after 2 weeks of incubation (Figure 2). Later, Eh decreased more slowly with some variations and reached approximately 110 mV after 11 weeks. pH of the medium increased during 31 days from 5.5 to 6.4 and then stayed constant until the end of experiment (Figure 3).

Fe content increased rapidly and continuously until the end of the experiment where it reached on an average 268 ± 27 $mg.L^{-1}$ (4.80 ± 0.48 mM). All the solubilized iron was under the ionic reduced form Fe^{2+}, apparently without occurrence of any detectable Fe complexed form. It was checked that soluble Fe^{2+} represented more than 85% of total reduced Fe determined after HCl extraction (Lovley & Phillips 1986). Less than 15% of reduced Fe was in the solid phase either adsorbed or precipitated. So, the extent of reduction was not underestimated a lot by measurement of soluble Fe^{2+}. During the first 21 days, iron solubilization rate amounted 0.2‰ $Fe_{tot}.d^{-1}$ and then decreased to 0.05‰ $Fe_{tot}.d^{-1}$. Iron dissolution by reduction concerned 0.74% Fe_{tot} after 11 weeks incubation (Table 2). This relatively low amount of solubilized Fe

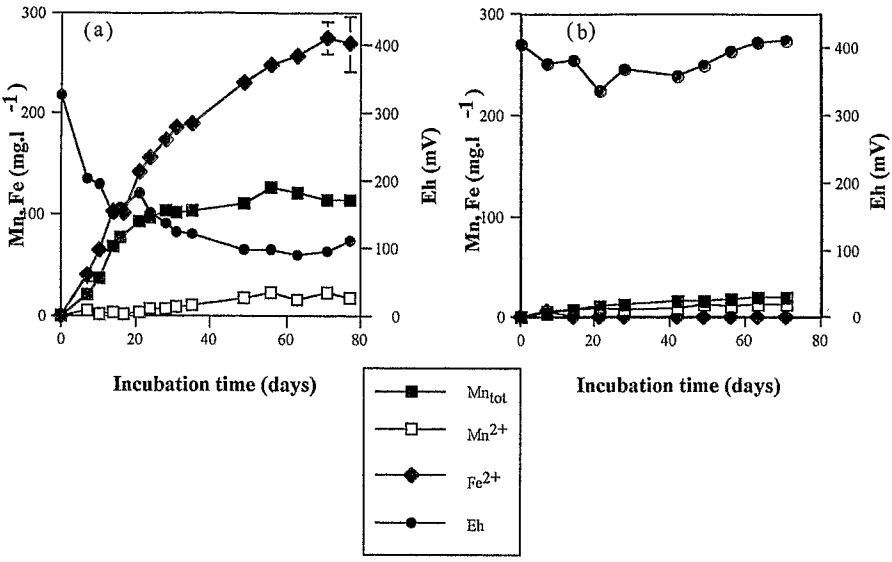

Figure 2. Fe and Mn concentrations in the medium in relation to Eh evolution and incubation time under biotic (a) and abiotic (b) conditions in experiments with glucose addition (GLU) (error bars not visible are smaller than symbols).

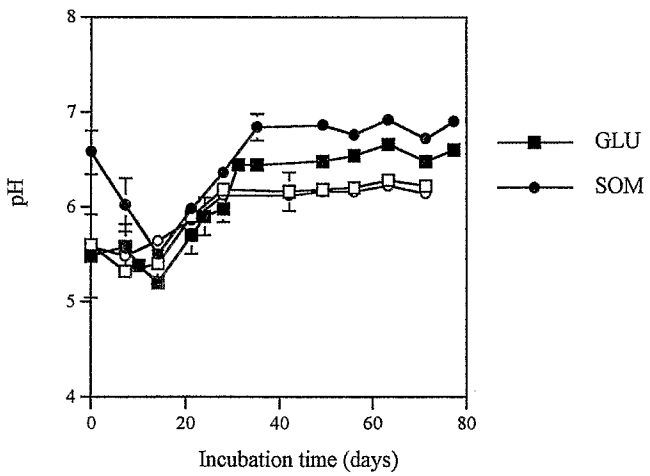

Figure 3. pH evolution in experiments with (GLU) and without (SOM) glucose addition, under biotic (filled symbols) and abiotic (open symbols) conditions.

Table 2. Percentage of maximum solubilization of Fe, Mn, Co and Ni in GLU and SOM treatments in biotic conditions.

Treatment	Fe_d/Fe_{tot}	Mn_d/Mn_{tot}	Co_d/Co_{tot}	Ni_d/Ni_{tot}
GLU	0.74	28	7	0.48
SOM	0.07	14	1	0.09

was sufficient to induce a major color change of the soil suspension from a yellow-red to a yellow-green color (results not shown).

Mn solubilization increased continuously during the first 24 days, reaching 97 ± 2 mg.L^{-1} (1.77 ± 0.04 mM). Then it stopped and a stationnary phase was noted. The proportion of total dissolved Mn (Mn_d/Mn_{tot}) was 28% of Mn_{tot} (Table 2). The average dissolution rate was 1.4% $Mn_{tot}.d^{-1}$ during the first 24 days. Mn^{2+} was observed only after 24 days. Then the Mn^{2+} concentration increased progressively to 22 ± 5 mg.L^{-1} (Figure 2). Free ionic Mn^{2+} represented less than 20% of Mn_d, suggesting that organo-metallic complexes constituted the major part of Mn_d.

Biotic treatment with autochthonous organic matter (SOM)

In the SOM treatment, Eh decreased gradually and continuously with time, reaching ca. 130 mV after 35 days (Figure 4). pH first decreased from 6.5 to 5.6 then increased to 6.8. It was always significantly higher than in GLU treatment (Figure 3).

With only autochthonous soil organic matter, Fe reduction was low. It reached 23.8 ± 6.4 mg.L^{-1} (0.43 ± 0.11 mM) after 11 weeks (Figure 4) corresponding to 0.07% Fe_{tot} (Table 2). This was 10 times less than with glucose addition. The solubilization rate changed after 50 days from 0.004‰ to 0.02‰ $Fe_{tot}.d^{-1}$. No significant change in color was observed.

Mn solubilization began earlier than for Fe and increased continuously until the end of experiment when it amounted to 14% Mn_{tot} (Table 2). Mn^{2+} in solution increased continuously during the first 28 days and then leveled off at approx. 10 mg.L^{-1} (0.18 mM). No difference occurred between biotic and abiotic treatments for Mn^{2+} in solution. Solubilized Mn^{2+} in controls corresponded to Na exchange from thimerosal.

Trace metal solubilization

Co and Ni solubilization was very low in controls (1% and 0.9‰ of Co_{tot} and Ni_{tot}, respectively) and were attributed to metal exchange with Na of the thimerosal (Figure 5). When bacterial activity was stimulated by glucose

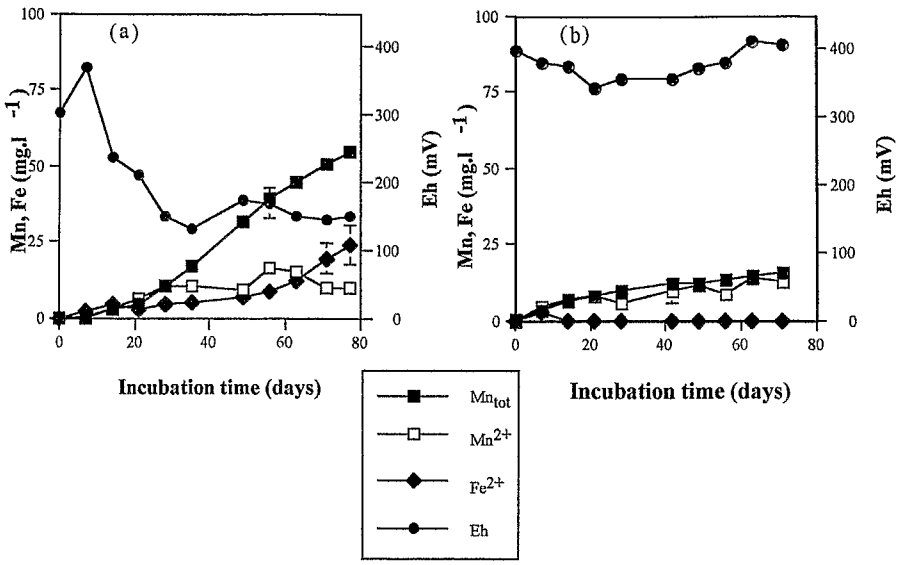

Figure 4. Fe and Mn concentrations in the medium in relation to Eh evolution and incubation time under biotic (a) and abiotic (b) conditions in experiments with only soil organic matter (SOM) (error bars not visible are smaller than symbols).

addition, Co behavior showed two steps: first a solubilization until day 24 that reached 7% Co_{tot} and then a slow decrease in solution (Figure 5(a)). For Ni, the same trend was observed with a maximum concentration occurring at day 14 (Table 2).

In treatments where only soil organic matter was available, the solubilization of Ni and Co was low and led to the dissolution of less than 1‰ Ni_{tot} and 1% Co_{tot} (Figure 5(b) and Table 2).The solubilization curves for Ni and Co *vs* time showed the same trend as in experiments with glucose addition. Dissolved Ni and Co increased during an initial phase, followed by a decrease during a second phase. Maximum of solubilization was reached after approx. 35 and 55 days for Ni and Co, respectively. However, these maxima were not well defined due to the very low concentrations.

Relationship between parameters

CO_2 production, i.e. carbon mineralization corresponding to bacterial activity, was highly correlated with all parameters except Ni. With additional glucose as complementary carbon source, Mn and Fe solubilizations were positively correlated during the entire experiment and more highly during the first 21 days (Table 3). After 3 weeks, no complementary Mn solubilization occurred whereas Fe solubilization continued. The solubilization of Co

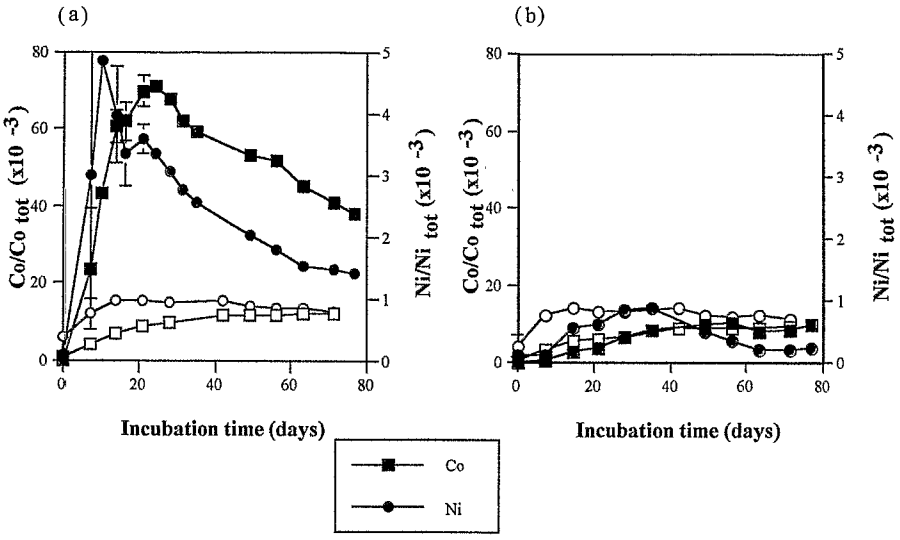

Figure 5. Trace metal solubilization rates during 77 days incubation in stimulated (a: GLU) and non-stimulated (b: SOM) experiments under biotic (filled symbols) or sterile (open symbols) conditions.

Table 3. Correlation matrix for 21 (a) and 77 (b) days of incubation for GLU treatment under biotic conditions.

(a)	FeII/Fe_{tot}	Mn/Mn_{tot}	Co/Co_{tot}	Ni/Ni_{tot}	(b)	FeII/Fe_{tot}	Mn/Mn_{tot}	Co/Co_{tot}	Ni/Ni_{tot}
C-CO_2	0.977**	0.966**	0.939**	0.545*		0.926**	0.929**	0.574**	−0.095
FeII/Fe_{tot}		0.968**	0.946**	0.589*			0.923**	0.478**	−0.202
Mn/Mn_{tot}			0.977**	0.603*				0.752**	0.054
Co/Co_{tot}				0.756**					0.615**
	$n = 24$					$n = 59$			

(* $p \leq 0.01$; ** $p \leq 0.001$).

closely followed that of Mn and Fe, and a highly significant correlation was found for Co *vs* Mn particularly for the first 21 days. Later, the decrease of Co in solution corresponding to the steady state level of Mn solubilization revealed a lower correlation. The same relationship was observed for Ni *vs* Mn, Ni *vs* Fe at a lower correlation level. For Co *vs* Fe during the first 21 days, the correlation was also high. There was no correlation between Ni and Fe for the entire 77 day incubation.

In experiments where autochthonous soil organic matter was the only source of carbon and energy available for bacteria, Mn and Co, and Mn and Fe solubilizations were correlated during the entire incubation (Table 4). Co

Table 4. Correlation matrix for 35 (a) and 77 (b) days of incubation for SOM treatment under biotic conditions.

(a)	$FeII/Fe_{tot}$	Mn/Mn_{tot}	Co/Co_{tot}	Ni/Ni_{tot}	(b)	$FeII/Fe_{tot}$	Mn/Mn_{tot}	Co/Co_{tot}	Ni/Ni_{tot}
$C\text{-}CO_2$	0.721**	0.978**	0.972**	0.907**		0.838**	0.990**	0.925**	−0.234
$FeII/Fe_{tot}$		0.681**	0.749**	0.814*			0.830**	0.605**	−0.238
Mn/Mn_{tot}			0.973**	0.874*				0.841**	−0.245
Co/Co_{tot}				0.944**					0.262
	$n = 24$					$n = 44$			

(* $p \leq 0.01$; ** $p \leq 0.001$).

solubilization was well correlated with Mn and Fe ones for 77 days. Positive correlations of Ni *vs* Mn and Fe were observed for the initial 35 days only. It can be underlined that correlation coefficients were higher between Fe and Ni, and Mn and Ni than in GLU treatment.

Discussion

The batch experimental approach was used to simulate mineral-micro-organism-organic interactions occurring in soil and to study the mobility and bioavailability of major and trace elements in terrestrial ecosystems particularly soil-plant systems. The results allowed to observe and establish relations between bacterial degradation of organic matter, bacterial reduction of Fe and Mn, and solubilization of trace metals, especially in anaerobic conditions.

Organic matter biodegradation was significant in biotic treatments under anaerobic conditions, though the C:N ratio seemed not particularly favorable for biodegradation. The addition of glucose increased C mineralization but the difference in CO_2 production between the GLU and SOM treatments corresponded to less than 5% of the added glucose. The main part of glucose (80% of added carbon) is transformed in fermentation products i.e. acetic, propionic and butyric acids and in CO_2. It appears that other factors than the C source was thus limiting the microbial activity. Eh decreased only under biotic conditions demonstrating that the reduction process was due to microbial activity, including respiration and fermentation processes. The kinetics of mineralization and Eh evolution vary with the nature of available C, because different types of metabolism are involved in microbial energy production depending on the type of available C. The production of organic acids in supernatants of the GLU treatment showed that acid fermentative bacteria were present as a large community, that can be significantly involved in the reduction process. On the other hand, the lack of such organic acids

in supernatants of the SOM treatment indicated that anaerobic respiration or other type of fermentation were the main processes involved in the reduction phenomena observed under these conditions.

The main mineral electron acceptors that permitted bacterial activity in these experiments were Mn(IV) and Fe(III) because NO_3^- and SO_4^{2-} were absent. As seen previously and to some extent, organic compounds can be used as other potential electron acceptors. Mn and Fe solubilization through this reduction process was a direct result of this anaerobic bacterial activity. Various types of bacteria are able to reduce MnO_2 and Fe_2O_3 and bacterial reduction of oxides in soils is considered as an important way of mobilization of non-available Mn or Fe (Ehrlich 1996). In this work, some bacterial strains able to reduce both Fe and Mn oxides have been isolated but not completely identified (Quantin et al. 1999). The observed phenomenon involved certainly large communities including fermentative and non-fermentative bacteria, as mentioned previously. In regard to the carbon mineralization rate, organic substrates do not have the same efficiency to promote bacterial Fe and Mn reduction. Glucose addition stimulates communities coupling glucose fermentation and Fe(III) reduction (Lovley 1987), and communities using metabolized compounds to reduce Fe(III) (Lovley & Phillips 1989; Markwiese & Colberg 2000). Communities using only SOM (i.e. humic like compounds) exhibit a lower yield to reduce Fe comparatively to the same amount of mineralized carbon. The quantity of Fe solubilized (moles of Fe n_{Fe}) per mole of released CO_2 (n_{CO_2}) was $n_{Fe}:n_{CO_2} = 0.0164 \pm 0.006$ in the SOM treatment. It was lower than in the GLU treatment ($n_{Fe}:n_{CO_2} = 0.1099 \pm 0.0298$). Mn solubilization (moles of Mn:n_{Mn}) per mole of evolved CO_2 differed less between the two treatments ($n_{Mn}:n_{CO_2} = 0.0468 \pm 0.0105$ and 0.0376 ± 0.0025 for GLU and SOM treatments after 11 weeks, respectively). It became not significant at the end of experiment. Glucose was thus a more efficient source of carbon for Mn- and Fe-reducing bacterial community than soil organic matter. Anaerobic oxidation of organic matter presents a very good correlation with Mn and Fe solubilization as proposed by some authors (Ehrlich 1987; Lovley & Phillips 1988; Lovley et al. 1989; Lovley 1991), and, as a consequence, in the weathering of oxides.

A large part of Mn was not under ionic form but appeared complexed in solution, especially when organic acids were produced by fermentation of glucose. Mn seemed to have a higher affinity for these compounds than Fe, which was present in solution under reduced ionic form only. Two explanations are possible. Either Mn^{2+} was produced before Fe^{2+} by biological reduction and organic complexes may have been formed with Mn^{2+} rather than with Fe^{2+}, as commonly observed. Alternatively, the labile biological

form Mn^{3+} may have been formed during the reduction process and this form has a higher affinity towards dissolved organic compounds than Fe^{2+}.

Mineralogical studies of this type of soil have shown the presence of large amounts of Fe oxides mainly goethite and traces of hematite as well as Mn oxides like asbolane (Nalovic & Quantin 1972; Schwertmann & Latham 1986; Quantin et al. 1997). The present results showed that Mn and Fe solubilization were closely correlated for the first 3 weeks when glucose was added to the medium and during the entire experiment when only SOM was provided. The kinetic data suggest that both Mn- and Fe-oxides were weathered during the first 3 weeks in GLU treatment and later only Fe-oxides were reduced. During the first phase, the amount of solubilized Mn was in the same order as that of Fe, though the total amount of Fe in the soil was 100 times greater than that of Mn. Solubilized Mn was relatively larger than solubilized Fe in the SOM treatment. This is in agreement with previous observations that have shown a preferentially reduction of Mn-oxides in reducing environments where Mn and Fe oxides coexist (Brümmer 1974; Ehrlich 1987). This is also to be expected from the higher reduction potential of Mn^{4+}/Mn^{2+} relatively to that of Fe^{3+}/Fe^{2+} (Madigan et al. 2000). The nature of the Fe-oxides reduced during the incubation could not be determined with certainty. However, two scenarios seem possible. First, acid NH_4-oxalate extractions showed the presence of amorphous and poorly crystallized Fe oxides in the sample (Becquer, unpublished results) corresponding to 17‰ of Fe_{tot}. This Fe compartiment can be the first to provide the easier reducible iron solubilized during the experiment. In fact, some studies have also shown that amorphous or poorly crystallized oxides of Fe and Mn are more readily reduced by microorganisms than highly crystallized minerals (Munch & Ottow 1980; Lovley & Phillips 1987; Francis & Dodge 1988; Ehrlich 1996). Secondly, the soil suspension yellowing observed during incubation with glucose supply can be explained by a preferential reduction of hematite over goethite as often noted in tropical soil observations (Barrón & Torrent 1987) and in experiments with chemical reactants (Jeanroy et al. 1991; Peterschmitt et al. 1996) or biological processes (Fischer 1988; Macedo & Bryant 1989). Several recent studies (Roden & Zacchara 1996; Urrutia et al. 1998) showed that specific surface is one of the main factor governing the availability of Fe-oxides to microbial reduction. In such a case, hematite having a smaller specific area is more recalcitrant than goethite. Nevertheless, different studies showed that Al substitution is also an important factor controlling oxide reduction. In this soil, as goethite is Al-substituted (Quantin et al. 1997), preferential hematite reduction over goethite can occur (Barrón & Torrent 1987; Torrent et al. 1987). The decrease of the rate of Fe solubilization with time could then be related to the progressive disappearance of

poorly crystallized oxide and/or hematite leading to the reduction of more recalcitrant minerals such as goethite or to the adsorption of Fe^{2+} on goethite surface (Urrutia et al. 1998).

The analysis of supernatants showed that solubilization of Ni and Co occurred simultaneously with the reduction of Mn- and Fe-oxides in biotic treatments, especially in GLU treatment. Mn and Fe reducing bacteria may well be indirectly responsible for the release of oxide associated metals (Ehrlich 1996; Bousserrhine et al. 1999). The observation of dissolution kinetics allows to investigate the nature of the association between Fe- Mn-oxides and Ni and Co. Correlation analysis showed that solubilized Co, and to a lesser extent Ni, were more closely related to solubilization of Mn than to Fe, for GLU treatments especially. Thus it can be argued that solubilized heavy metals were mainly associated to Mn-oxides, as often observed for Co in minerals like asbolane and lithiophorite (Taylor 1968; McKenzie 1989). Poorly crystallized Fe-oxides are first reduced but the change in color suggests that Fe-oxides like hematite can be reduced too during this first period. Hematite is generally little substituted and goethite less reducible even when it was substituted (Gasser et al. 1996; Bousserrhine et al. 1998, 1999). A dominance of both types of minerals could thus explain the low correlation between Fe and heavy metals. Such considerations and the results suggest that bacterial reduction and weathering concern a mixed oxide of Mn, Co and Ni like asbolane and/or lithiophorite. This minoritary mineral phase seems to control trace metal mobility and release. On the opposite, the large amount of Fe-oxides in the soil seems to be less important as a source of trace metals. When the autochthonous organic matter is the only source of carbon, reduction of oxides leads to the release of significant quantities of Mn and Fe but seems to have a small effect on Co and Ni solubilization. Nevertheless, in the solid phase, metal speciation evolves to the more labile compartments (results not shown).

Insolubilisation steps were observed by the decrease of Co and Ni concentration in solution, but the involved amounts were very low. The metals may be adsorbed on the surface of residual mineral or organic phases or precipitated together in a residual mineral. Co and Ni can also be adsorbed on non-weathered oxides like goethite, particularly under neutral pH conditions as in the present experiment. This phenomenon even leads to incorporation of metals in the mineral lattice (Brümmer et al. 1988). Such adsorption may decrease the accessibility of the sites for bacterial Fe reduction and decrease the Fe solubilization rate (Bousserrhine et al. 1998). Cooper et al. (2000) observed that microbial reduction of oxides could enhance the rate of oxide recrystallization and consequently act to immobilize heavy metals. Moreover, sequential extractions (Leleyter & Probst 1999, modified) performed on

residual samples showed that the quantity of Co associated to exchangeable and poorly crystallized Fe-oxide compartments increased a lot after incubation in both treatments (results not shown). Ni became also more associated to the poorly crystallized Fe-oxides, especially after GLU treatment. But it remains difficult to distinguish the part due either to weathering, or precipitation or adsorption. Similarly, metals can be adsorbed on soil organic matter and/or microbial biomass. It is well known that bacteria and fungi can provide sites for metal sorption and neoformation of minerals (Beveridge et al. 1983; Mullen et al. 1989).

Conclusion

The results of our study showed that a mixed and labile Mn-oxide like asbolane and/or lithiophorite can be a source of easily mobilizable Mn, Co and Ni involving bacterial reduction processes. Fe-oxides, which are mainly present as well crystallized goethite, were solubilized after Mn-oxides, and were probably a secondary source of trace metals. Mn- and Fe-reducing bacteria were responsible for this solubilization in association with anaerobic respiration or fermentation processes. The nature and the availability of organic carbon compounds, used as nutrients, regulated the intensity of these processes. The increased bioavailability of metals like Mn, Co and Ni, especially in case of organic input, may induce toxicity for crop plants. The observed adsorption or precipitation phenomena following dissolution processes can counteract this effect by limiting the availability of these metals.

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