Impact of nitrogenous fertiliser-induced proton release on cultivated soils with contrasting carbonate contents: A column experiment

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Abstract

An experimental study was carried out in order to evaluate the impact of nitrogen fertiliser-induced acidification in carbonated soils. Undisturbed soil columns containing different carbonate content were sampled in the field. Fertiliser spreading was simulated by NH_4Cl addition on top of the soil column. Soil solution composition (mainly nitrate and base cations) was studied at the soil column's base. Nitrification occurred to a different extent depending on soil type. Higher nitrification rates were observed in calcareous soils. In all the soil types, strong correlations between leached base cation and nitrate concentrations were observed. Regression coefficients between base cations, nitrate and chloride were used to determine the dominant processes occurring following NH_4Cl spreading. In non-carbonated soils, nitrogen nitrification induced base cation leaching and soil acidification. In carbonated soils, no change of soil pH was observed. However, fertilisers induced a huge cation leaching. Carbonate mineral weathering led to the release of base cations, which replenished the soil exchangeable complex. Carbonated mineral weathering buffered acidification. Since direct weathering might have occurred without atmospheric CO_2 consumption, the use of nitrogen fertiliser on carbonated soil induces a change in the cation and carbon budgets. When the results of these experiments are extrapolated on a global scale to the surface of fertilised areas lying on carbonate, carbonated reactions with N fertilisers would imply an additional flux of 5.7×10^{12} mol yr^{-1} of Ca + Mg. The modifications of weathering reactions in cultivated catchments and the ability of nitrogen fertilisers to significantly modify the CO_2 budget should be included in carbon global cycle assessment.

1. INTRODUCTION

Nitrogen fertilisers are widely used in most agricultural areas. N fertilisers constitute 60% of the 154 and 4 million tons of fertilisers (N + P_2O + K_2O) spread on soils in 2006–2007 in the world and in France, respectively (FAO, 2008). The world average annual increase in fertiliser consumption was 3.3% from 1961 to 1997 and prediction from FAO's study is a 1% increase per year until 2030 (FAO, 2008).

The impact of nitrogen fertiliser includes leaching of NO_3 to surface and ground water and eutrophication (e.g. Seitzinger and Kroeze, 1998). Another consequence of the use of fertilisers is the release of protons in the soil during the nitrification process. N fertiliser oxidation produces two protons for every nitrified ammonium ion (e.g. Berden and Nilsson, 1996; Barak et al., 1997; Tarkalson et al., 2006). Fertiliser spreading has been shown to induce soil acidification and the loss of base cations in the context of field studies (Stumpe and Vlek, 1991; Poss and Saragoni, 1992; Cahn et al., 1993; Nohrstedt, 2001; Frank and Stuanes, 2003; Pierson-Wickmann et al., 2009) and laboratory experimental settings (Faurie, 1977; Berthelin et al., 1985;

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Mochoge and Beese, 1986; Berden and Nilsson, 1996). These studies mostly focussed on acidic and forest contexts. Carbonate substrata provide worldwide high-grade agricultural soils, especially adapted for crop production, and represent 47% of the total cultivated areas on Earth. Consequently, the related soils are located in areas where fertilisers are the most widely spread.

Protons released by nitrification represent an anthropogenic acidification process. Few studies have dealt with the impact of anthropic induced acidity on silicate weathering processes, nutrient losses (Van Breemen et al., 1984; Paces, 1985; Reuss et al., 1987; Falkengren-Grerup et al., 1987; Wright et al., 1988; Probst et al., 1992, 1995, 1999; 2000; de Vries et al., 2003) and CO_2 consumption (see for example Amiotte-Suchet et al., 1995), but the carbonates were not generally of interest because of their high buffering capacity. However, carbonate rocks are highly weatherable and constitute major CO_2 assimilators. The carbonate bedrocks represent about 15% of total continental surfaces, but their weathering releases two times more carbon in rivers than that released by silicate outcrops (e.g. Gaillardet et al., 1999; Amiotte-Suchet et al., 2003; Lerman and Wu, 2007). The enhanced carbonate weathering by anthropogenic acidity inputs has to be considered because the modifications in weathering rates might influence the element fluxes to riverine systems and, consequently, river and ocean ecology as well as the carbon cycle.

The proton inputs to carbonated or aglimed areas, as generated during nitrification of N fertilisers, have been shown to affect the chemical composition of rivers, especially Ca^{2+} and HCO_3^- concentrations (Probst, 1985; Etchanchu and Probst, 1988; Semhi et al., 2000; Raymond and Cole, 2003; Oh and Raymond, 2006; Hamilton et al., 2007; Perrin et al., 2008). At the catchment level, this acidity input has been shown to participate in carbonate mineral weathering, potentially leading to direct and indirect CO_2 releases to the atmosphere (Perrin et al., 2008). The contribution of atmospheric/soil CO_2 to riverine alkalinity was estimated to decrease by about 7–17% on average in catchments draining carbonates and receiving N fertilisers. This anthropogenic CO_2 source could reach more than 6-15% of the annual CO_2 uptake by natural silicate weathering and could, consequently, partly counterbalance this natural CO_2 sink (Perrin et al., 2008). In this context, the impact of proton release during N-fertiliser nitrification processes on calcareous agricultural soils, observed at the catchment level, needs to be investigated at the level of the soil to highlight the related mechanisms. Although soil column experiments are a convenient way to investigate soil processes, in a controlled environment, soil weathering laboratory studies are scarce.

This study proposes to investigate the impact of nitrogen fertilisers on a range of calcareous content cultivated soils, with a laboratory experimental approach using soil columns. The goals of this study are:

(i) to evaluate the consequences for soil and soil solution chemistry of nitrogen spreading on cultivated carbonated and non-carbonated soils;
(ii) to investigate the processes occurring after nitrogen fertiliser inputs on soils and during the nitrification

process in soil by studying the soil solution composition and the ratio between elements;
(iii) to quantify the increase in soil cation leaching resulting from enhanced carbonate dissolution in fertilised areas and to evaluate the consequence for the CO_2 budget.

2. MATERIALS AND METHODS

2.1. Soils and area description

The studied soils are from the Montoussé catchment, which is located in the southwest of France in the geomorphologic area of the "Coteaux de Gascogne" (Perrin et al., 2008). The climate in this area is oceanic with an average temperature of 12.7 °C (minimum temperature in January is 5 °C, on average and maximum temperature in August is 20 °C, on average). The mean (average from 1988 to 2004) annual precipitation and evapotranspiration are about 667 mm and 871 mm, respectively. The dry period runs from June to August (months with the maximum water deficit) and the wet period is from October to May (Ribeyreix-Claret, 2001).

The studied area lies on a molasse bedrock as defined by Cayeux (1935), Crouzel (1957) and Revel (1982). Clays, sandstones, limestones and marl mix in banks, with a continuous lateral passage. For further description of the area, see (Perrin et al., 2008). Scanning electron microscopy (SEM) and electron microprobe phase analyses were performed on thin soil sections on both carbonated and carbonate-free soil profiles. Quartz (mainly), but also feldspar (plagioclase and albite), calcite, zircon and less commonly monazite, chlorite, epidote, ilmenite, mica (muscovite), apatite and tourmaline were detected. Calcite is mainly Mg–Calcite with on average $96.3 \pm 1.8\%$ Ca and $0.6 \pm 0.5\%$ Mg as performed by electron microprobe phase analyses. The respective proportion in weight of the total major elements composition for (Ca + Mg), (Na + K) and (Fe + Mn) were $36 \pm 20\%$, $14 \pm 5\%$ and $16 \pm 7\%$, respectively, in molasse bedrock as determined by ICP-OES analyses after acid dissolution (HF + HNO_3) and oxidation by H_2O_2 (Bur et al., 2009). Concretions composed of a clay-like matrix mixed with manganese and iron oxides are commonly found in soils. X-ray diffraction (XRD) analyses on 2 mm sieved soils and oriented slides of the clay-like matrix indicated that carbonate and/or smectite were dominant (depending on the carbonate content of the soils), followed by illite and vermiculite (in similar proportions) and kaolinite.

The Montoussé catchment is representative of the geomorphology and land use in this area: 90% of the area is cultivated with wheat and sunflowers in rotation. Farmers spread principally chemical fertilisers on soils: 60% as ammonitrate (NH_4NO_3), 35% as compound fertilisers of the N–P–K type (N, P_2O_5 and K_2O), 3% as urea, and 2% as straight fertilisers such as sulfonitrate (($NH_4)_2SO_4$). The usual amounts of chemical fertilisers spread in this area are as follows: 670 kg ha^{-1} yr^{-1} for wheat (comprising 30% N, 5% P_2O_5 and 2% K_2O), and 400 kg ha^{-1} yr^{-1} for sunflowers (comprising 22% N, 4% P_2O_5 and 5% K_2O).

2.2. Sampling of soil columns

The influence of fertiliser inputs on soils was evaluated with laboratory experiments. Three soils from the Mont-oussé catchment originating from the same sedimentary bedrock (molasse type), were chosen for their range of carbonate contents, in order to get a gradient from carbonate-free soils to saprolite carbonate level.

Samples were collected on February 2007. Acid-washed polypropylene columns ($H = 5$ cm, $\varnothing = 4,2$ cm) were gently pushed into the soil, in order to limit soil disturbance and preserve the soil structure as much as possible. After soil sampling, a perforated piece of PTFE (washed with a dilute acid solution and deionised water) was embedded at the bottom of the column to prevent soil loss. This system was maintained to fit on plastic tubes used for the collection of leachates (Fig. 1).

Three soil types with different carbonate contents were sampled. For each soil, three columns were collected. Soil columns were extracted from the sub-surface carbonated layer (Carb. type) and the molasse saprolite layer (Mol.$_{Sap}$ type) in a carbonate soil profile (calcic cambisol soil – World Reference Base, 2006), at 5–10 cm and 100–105 cm, respectively. This soil was located on a characteristic middle slope in the lower part of the catchment. Carbonate-free (Carb. free type) soil columns were collected at the sub-surface (5–10 cm) of a carbonate-free (or carbonate leached) soil profile (calcic luvisol – World Reference Base, 2006) located on the same slope about 400 m SE of the carbonated soil profile. Soils columns were kept in the dark and at room temperature during the following experiments to reproduce field conditions. Physico-chemical characteristics of sampled soils are summarised in Table 1.

2.3. Experimental design

2.3.1. Soil column treatments and leaching experiments
2.3.1.1. Nitrogen inputs. A solution of ultrapure NH$_4$Cl diluted in ultrapure water was added on the surface of the experimental soil columns to simulate nitrogen fertiliser application to soils. Chloride ions, whose conservative behaviour has already been observed in soils (Hornung et al., 1990), were introduced in soil columns simultaneously with NH$_4$, and were used as a tracer. For each soil type, a column sample was kept as a reference during the

Table 1
Physical and chemical characteristics of the sampled soils.

	Units	Soil 1 Carb. free	Soil 2 Carb.	Soil 3 Mol.$_{Sap}$
Depth	cm	5–10	5–10	100–105
pH water	–	6.6	8.2	8.5
pH KCl	–	5.9	7.6	8.2
CEC	cmol(+) kg^{-1}	25.4	19.3	15.9
Total carbonate content	%	0.1	15.3	50.6
Active carbonate content		–	3.8	11.9
Organic matter		2.3	2	0.4
C/N (masses ratio)	–	9	8.3	6.3
Bulk density	g cm^{-3}	1.33	1.72	1.52
Texture	%			
Sand (50 μm to 2 mm)		18	25.9	17
Silt (2–50 μm)		37.2	36.9	55.6
Clay (<2 μm)		44.8	37.2	27.4

first part of this study and was leached with ultrapure water (Fig. 2).

Experiments were carried out in two steps:

(1) during the first part of the experiment, which will be referred to as "first treatment", two replicates were treated with dissolved NH$_4$Cl and a reference received ultrapure water;
(2) during the second part (called "second treatment"), the three sets of three columns received dissolved NH$_4$Cl amendments.

The amount of NH$_4$Cl in the first treatment was adjusted in order to simulate realistic application of NH$_4^+$ in fertiliser (310 kg N ha^{-1} a^{-1}). In the second NH$_4$Cl treatment, NH$_4^+$ inputs were two fold higher (610 kg N ha^{-1} a^{-1}) on the same columns in order to evaluate the potential extreme impact of

Fig. 1. Soil column devices with an exploded diagram.
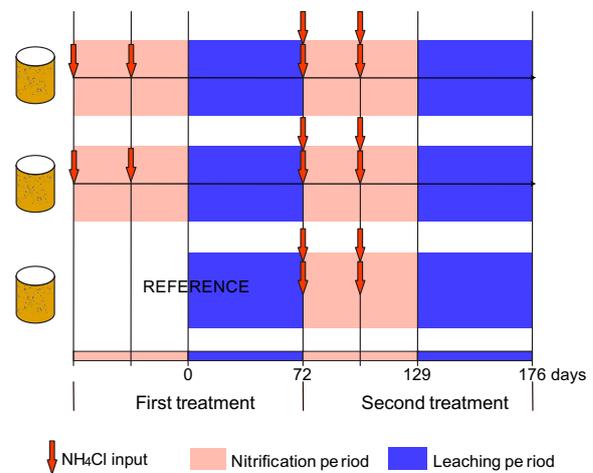
Fig. 2. Experimental design for each soil type: two of the three soil column replicates received the same treatment and one column was used as a reference in the first step of the experiment.

fertiliser inputs. For both treatments, NH_4Cl was applied twice (half of the total amount each time with a 10 day interval between applications) (Fig. 2). The first inputs aimed at stimulating soil bacteria (Faurie, 1977; Mochoge and Beese, 1986).

2.3.1.2. Leaching experiments. During the experiment, the columns were kept in the dark and the leaching experiments were carried out at room temperature (20 ± 2 °C) to match optimum field conditions. The leaching period of the experiments began 14 days after the second fertiliser application (Faurie, 1977; Thomson et al., 1993). All columns were leached for 72 days for the first treatment and 46 days for the second one (Fig. 2). Daily, 5 mL ultrapure water was gently added on top of the columns. This corresponded to around 3.6 mm days^{-1} of precipitation (i.e. similar to a rainy spring with about 100 mm per month in the studied area). Soil solution leachates were collected at the bottom of the columns. Water circulation inside the soil column was unimpeded to simulate field conditions; therefore, the amount of water collected each day was variable.

2.4. Analytical methods

2.4.1. Solutions

Water samples collected in polypropylene tubes were filtered in the laboratory through a 0.22 μm Millipore nitrocellulose filter, stored in the dark at 4 °C and analysed quickly. Major cations were analysed on acidified samples by atomic absorption spectrometry (AAS 5100 PC, Perkin–Elmer) after adding La (0.5%) for Ca^{2+} and Mg^{2+} analyses. Major anion (NO_3^-, Cl^- and SO_4^{2-}) and NH_4^+ concentrations were measured by high performance liquid-ion chromatography (Dionex Chromatograph ICS 2000, Dionex Co., Sunnyvale, CA, USA). In all cases, the standard ranges were checked using the international standard NWRI-ION-915 and the errors were minor at 5%. When a sufficient amount of water had been collected, alkalinity was determined by titration with HCl acid (DMS Titrino, Metrohm). The error was always less than 3%.

2.4.2. Soils

The total carbonate content of the soil before the experiment (corresponding to the amount of CO_2 released from the soil aliquot in the presence of HCl) and the active calcium carbonate content (calcium carbonate not soluble in ammonium oxalate, see Callot and Dupuis, 1980), were measured at the Laboratoire d'Analyse des Sols (http://www.arras.inra.fr/, INRA, Arras, France) in accordance to the norms ISO 10693 and AFNOR NF X 31-106, respectively (Callot and Dupuis, 1980; AFNOR, 1996).

After the experiments, each soil column was divided into three sub-samples: surface, middle, and bottom of the column corresponding to 0.8, 2.5 and 4.2 cm mean depths. For physical and chemical measurements, soil samples were air-dried and sieved through a 2 mm grid. The pH in a 1:5 soil/H_2O suspension (pH_w) (ISO 10390) and soil/1 M KCl suspension (pH_{KCl}) (ISO 10390) was measured for all soil samples, before and after leaching experiments following gentle shaking for 2 h (AFNOR, 1996). pH was measured

a second time after 24 h of shaking to control pH evolution. Only slight variations (<2%) were observed for 90% of the samples and variations never exceeded 5%. On the same soil samples, exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+, and Mn^{2+}) were measured by mixing 3 g of soil with 20 mL 0.1 M $BaCl_2$ solution, followed by shaking for 2 h (Hendershot and Duquette, 1986). Exchangeable cations were analysed by ICP-OES (Thermo IRIS INTREPID II XDL, Waltham, MA, USA).

Uncertainties for these analyses were checked by using soil replicates. The standard deviation is indicated when the calculation was possible.

For the purposes of clarity, charges (except for H^+) are omitted in the rest of the manuscript.

3. RESULTS

3.1. Soil leachates

3.1.1. Elemental concentrations

Mean concentrations of major cations (Ca and Mg), alkalinity when measured and nitrogen species in leachates of the nine studied columns for the two treatments are presented in Table 2. During the first treatment, lower concentrations of all solutes were measured for the reference columns. NH_4 concentrations were measured only for a few samples and were found to be lower than the quantification limit (25 μg l^{-1}). The highest concentrations of base cations and of nitrates were measured in the molasse soil leachates and for the carbonated soils, respectively. During the second treatment, both carbonate and molasse soil leachates showed the highest base cation and nitrate concentrations, whereas the highest NH_4 concentrations were measured in the carbonate-free column leachates. The alkalinity could be measured in some leachate samples from the carbonated and molasse soils. No clear trend could be observed, although concentrations remained very low during the experiments. Measured concentrations in leachates were highly variable as can be seen by the min-max values. Concentrations in leachates varied during experiments, but showed similar temporal trends, as can be observed in Fig. 3, where cumulative fluxes are plotted versus time.

3.1.2. Trends of element fluxes over time
3.1.2.1. Nitrogen leaching. The nitrification extent of NH_4 to NO_3 can be evaluated by NH_4 and NO_3 concentrations in leachates and by the amount of NO_3 leached out of the columns compared to the amount of NH_4 brought to the columns (Table 2). The cumulative fluxes of NO_3 provide temporal information about this process (Fig. 3).

Nitrates were detected in the reference column leachates, indicating that the sampled soils contained some nitrates. In the reference carbonate and carbonate-free soils (Fig. 3a and b), the cumulative nitrate fluxes were low (0.14 and 0.11 mmol, respectively), whereas they were higher (0.33 mmol) for the molasse soils (Fig. 3c). The nitrates leached from the reference columns revealed their natural occurrence in cultivated soils, in relation with agricultural practices. Even if nitrates are easily leached anions, increased nitrate concentrations in soils after fertilisation

Table 2
Mean, minimal and maximal concentrations of base cations, nitrates, ammonium, alkalinity measured in soil leachates, and nitrification rate (ratio between ammonium input to leached nitrates).

		Treatment 1					Treatment 2				
		$Ca + Mg$	NO_3^-	NH_4^+	HCO_3^-	NH_4 nitrification to NO_3%	$Ca + Mg$	NO_3^-	NH_4^+	HCO_3^-	% NH_4 nitrification to NO_3
		Mean (min-max) in $mmol\,l^{-1}$					Mean (min-max) in $mmol\,l^{-1}$				
Carb.free	Col 1. Ref.	0.7 (0.14-1.88)	1.0 (0.16-0.85)	n.m.	n.m	–	18.6 (0.9-47.2)	5,3 (3.1-8.4)	16.1 (6-37.6)	n.m	12
	Col 2	8.4 (2.8-15.9)	6.1 (2.8-10)	n.m.	n.m	64	16.7 (0.1-90.7)	2.7 (1.3-8.2)	18 (5.5-52.5)	n.m	17
	Col 3	6.3 (0.9-18)	4.2 (0.1-7.8)	n.m.	n.m	50	22.6 (8.7-42.0)	3.3 (1.8-6.1)	18.4 (13.3-26.2)	n.m	14
Carb.	Col 1. Ref.	0.93 (0.76-1.05)	0.84 (0.28-1.13)	<d.l.	0.87 (0.45-20.5)	–	23.4 (2.5-80.7)	17.3 (1.6-42.6)	0.3 (0-1.8)	0.75	30
	Col 2	19.1 (2.1-39.6)	21.6 (1.5-46.5)	<d.l.	0.95 (0.37-1.87)	72	41.1 (8.2-94.2)	24.5 (8.1-41.9)	5.6 (0.1-26.1)	0.35 (0.21-0.52)	38
	Col 3	39.7 (8.7-58.9)	29.0 (7.3-100.7)	<d.l.	1.18 (1.17-1.19)	100	44.2 (5.2-90.3)	32.8 (4.5-90.3)	3.6 (0-13.2)	0.37 (0.24-0.55)	49
Mol.Sap	Col 1. Ref.	2.01 (0.92-3.97)	2.23 (0.79-6.01)	n.m.	1.151 (0.98-1.3)	–	26.8 (1-116.2)	3.6 (1.4-6.4)	12.5 (5.5-21.5)	0.45	11
	Col 2	31.5 (4.5-61.6)	7.95 (2.72-6.54)	n.m.	0.594 (0.50-0.76)	56	50.0 (25.6-76.9)	22.8 (13.5-39.8)	7.1 (1.1-10.9)	0.7	28
	Col 3	26.7 (10.4-42.1)	7.8 (1.4-19.5)	n.m.	0.66 (0.57-0.74)	50	127.1 (22.4-262.5)	45.6 (8.7-73.2)	7.4 (5.5-15.5)	1.06	25

Ref.: Reference column; Col.: column; d.l.: detection level; n.m. not measured.

can last for one year (Ruiz et al., 2002). Differences between the reference columns reflected field heterogeneity as well as nitrate behaviour in the catchment. The relatively high values of nitrates measured in the molasse saprolite reference compared to the other reference columns could be the consequence of nitrates leaching into the deep layers and of their low transformation related to reduced microbial activity. Nevertheless, cumulated fluxes of nitrates were low. For molasse soils, leaching volumes were low compared to the other soil types, due to difficult water circulation. Microbial activity seemed to be reduced in the molasse saprolite samples. Indeed, nitrate production did not occur at the beginning of the leaching experiment as nitrates fluxes increased only by the 40th day of the first experiment and remained moderate during the entire experiment (Fig 3c).

During the first treatment, the nitrification rate was above 50% in every column. In the carbonated soil columns, nitrification was almost complete, since the amount of NO_3 leached from the column after the first experiment reached 72% (col. 2) to 100% (col. 3) of the nitrogen inputs. During the second experiment, nitrification rates were lower for each column (only 12–49% of the total nitrogen input was leached as nitrates). Significant NH_4 concentrations were measured in all column leachates, reaching an average of 18.4 mmol l^{-1} in the carbonate-free soil columns (Table 2).

With the exception of the carbonate-free soil, replicate columns of the same soil type did not behave in exactly the same way, as a result of water circulation, microbial activity and heterogeneity in nitrification capacity in soil. Despite soil heterogeneity, the specific behaviours of each soil type could be observed.

3.1.2.2. Cations leaching. Calcium was the main base cation in all leachates: Ca and Ca + Mg constituted more than 90% and 95% of positive charges in leachates, respectively. The influence of nitrogen inputs on base cation leaching was obvious, since leachates from fertilised columns were much more concentrated than those from reference columns (Table 2 and Fig. 3 d, e and f). During the first treatment, the amount of leached calcium was 6-, 10- and 12-fold higher in molasse saprolite, carbonated and carbonate-free soils, respectively, if compared to reference columns. During the second treatment, the levels of Ca and Mg in leachates were about 1.5-fold higher than the amounts leached during the first treatment. Considering the amount of leached Ca, soil types can be classified as follows: carbonated soil > molasse > carbonate-free soil. Similarly to what was observed for nitrogen species, base cations fluxes were slightly different between replicates. However, it is worth noting that base cations fluxes showed similar patterns as nitrate fluxes in all replicates of amended soil samples for all soil types and for the two treatments.

3.1.3. Correlations between element fluxes in soil leachates

The trends in element fluxes as a function of time as described above (Fig. 3) clearly indicated that base cations and nitrates where leached simultaneously from the soil columns. The relationships between fluxes of base cations, chloride and nitrates (produced from NH_4 transformation) in leachates were thus considered. Fig. 4 shows the trend of
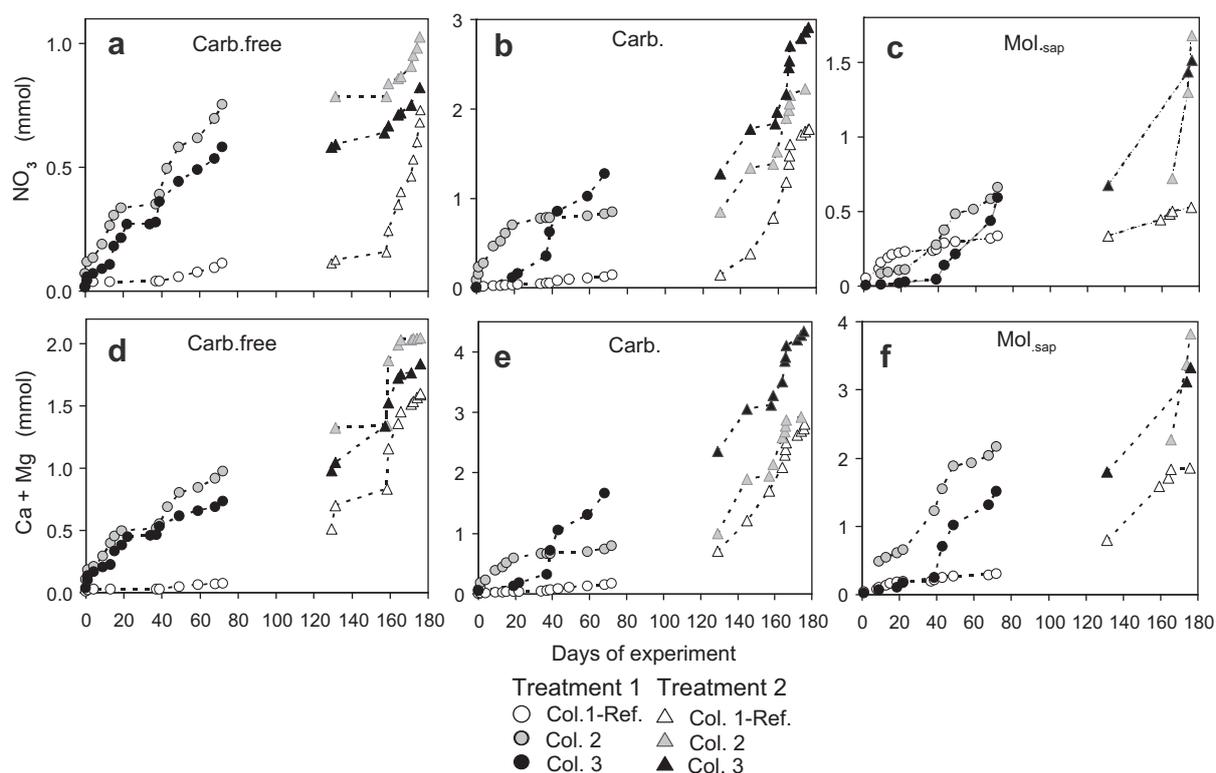
Fig. 3. Cumulative (Ca + Mg) and NO₃ fluxes (in mmol) in the leachates of carbonate free, carbonate and molasse saprolite soil columns over the time of experiment. The first experiment last from day 0 to day 72 and the second experiment from day 129 to day 176. (a) and (d): carbonate free soil; (b) and (e): carbonated soil; (c) and (f): molasse soil.

the leached (Ca + Mg) fluxes as a function of NO_3 (Fig. 4 a, b and c) and Cl (Fig. 4 d, e and f) fluxes. Linear regressions between base cations (Ca + Mg) and Cl as well as NO_3 fluxes for the different soil types and for each treatment are given in Table 3.

For amended columns, (Ca + Mg) amounts in leachates were both correlated to Cl and NO_3 amounts (Fig. 4). The slopes of the relationships depended on the soil type (Table 3). For each soil type, similar trends were observed for the replicates, except for the regression of Ca + Mg vs. NO_3 for carbonate-free samples during the second treatment.

For carbonate-free soils (Fig. 4a and d), different relationships between (Ca + Mg) and NO_3 were observed for the two treatments. The average slope was one during the first treatment, whereas no clear trend appeared during the second treatment. Indeed, in the first column (white triangles), there was no correlation. For the other two columns (grey triangles), the slopes were greatly variable (11.2 and 5.4). Conversely, correlation coefficients between (Ca + Mg) and chloride fluxes were similar (average value of 0.59) for all the treatments (Fig. 4d). Carbonated soil columns had a more stable behaviour (Fig. 4b and e). Regression correlation slopes between base cations and nitrates had an average value of 1.36, increasing between treatment 1 and treatment 2 (1.28 in the first treatment and 1.44 in the second treatment). For chloride, regression slope coefficients were close to one in both treatments. For molasse

saprolite samples (Fig. 4c and f), the relationships between base cation and NO_3 fluxes had even a higher slope coefficient, but the measurements were more variable depending on replicate columns (average of 1.66). The slope of Ca + Mg *versus* Cl relationship was 0.7 on average in both treatments (0.75 in the first treatment and 0.64 in the second treatment).

3.2. Changes in soil chemistry

3.2.1. Soil pH

Soil pH (in water and KCl, 1 N) as well as the amount of exchangeable cations were measured before and after the experiments at three depths of the soil columns (upper part, middle and bottom, corresponding to 0.8, 2.5 and 4.2 cm average depths). The results (average of the three columns) are shown in Fig. 5.

For carbonated soil and molasse saprolite samples, pH values did not change after the experiment. Conversely, in carbonate-free soils, pH showed a mean loss of 1.3 units. The pool of exchangeable protons in soils was calculated by considering the difference between pH in water and pH in KCl 1 N. In carbonate-free soil samples, the exchangeable H^+ contents after the experiment were 13-, 19- and 39-fold higher at depths of 0.8 cm, 2.5 cm and 4.2 cm, respectively. In the carbonated soil, pH and exchangeable acidity in the soil columns were not significantly modified after the
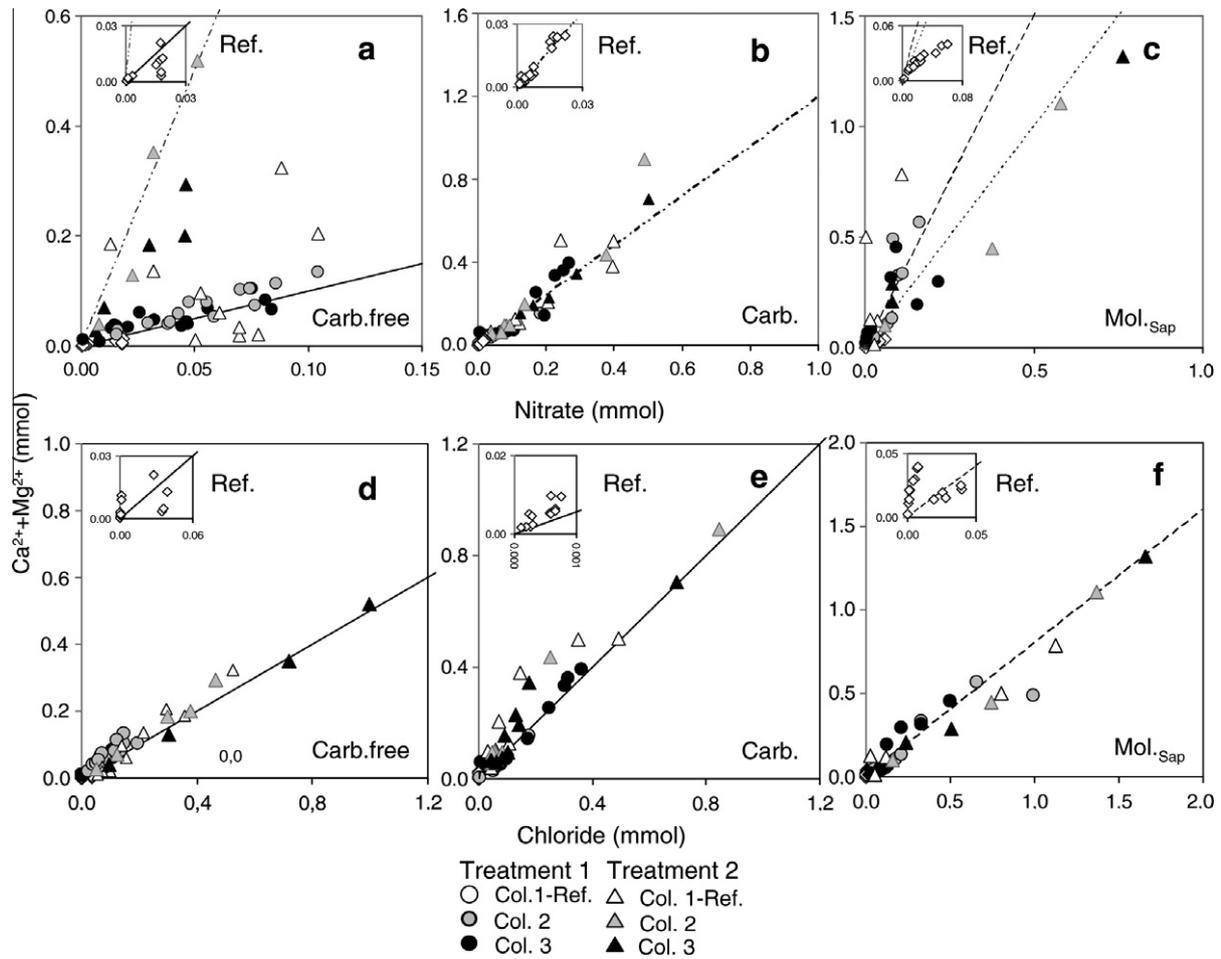
Fig. 4. Relationship between loads in mmol of (Ca + Mg) and NO₃, and (Ca + Mg) and Cl and in leachates of carbonate free (a) and (d), carbonated (b) and (e) and molasse saprolite soil (c) and (f) columns during the first and second treatments for reference and treated soil columns.

Table 3
Regression equations with the determination coefficient and the significance level of the relationships between fluxes of (Ca + Mg) and NO₃, (Ca + Mg) and Cl in mmol for carbonate free, carbonated and molasse saprolite soil columns during the first and second treatments for reference and treated soil columns.

			Ca + Mg = f(NO₃) mmol	Ca + Mg = f(Cl) mmol
Carbonate free soil	Reference	$n = 11$	$y = 0.55x + 0.001, r^2 = 0.51^*$	N.S.
	Treatment 1	$n = 30$	$y = 1.04x + 0.010, r^2 = 0.80^{***}$	$y = 0.63x + 0.009, r^2 = 0.83^{***}$
	Treatment 2	$n = 19$	N.S.	$y = 0.54x - 0.002, r^2 = 0.96^{***}$
Carbonated soil	Reference	$n = 17$	$y = 1.26x - 0.001, r^2 = 0.95^{***}$	$y = 9.86x + 0.001, r^2 = 0.78^{***}$
	Treatment 1	$n = 22$	$y = 1.28x - 0.013, r^2 = 0.86^{***}$	$y = 1.05x + 0.006, r^2 = 0.97^{***}$
	Treatment 2	$n = 27$	$y = 1.44x - 0.028, r^2 = 0.89^{***}$	$y = 1.02x + 0.050, r^2 = 0.91^{***}$
Molasse saprolite soil	Reference	$n = 14$	$y = 0.57x - 0.013, r^2 = 0.90^{***}$	N.S.
	Treatment 1	$n = 19$	$y = 1.99x + 0.062, r^2 = 0.53^{***}$	$y = 0.75x - 0.011, r^2 = 0.97^{***}$
	Treatment 2	$n = 11$	$y = 1.45x + 0.170, r^2 = 0.75^{***}$	$y = 0.64x + 0.034, r^2 = 0.79^{***}$

N.S. no significance, $P > 0.05$.
* $P \leqslant 0.05$
*** $P \leqslant 0.001$

treatments. The H⁺ content in molasse saprolite samples was only slightly modified at the surface of the soils; the exchangeable H⁺ pool was only 0.9-fold higher on top of the columns and was not modified deeper in the column.
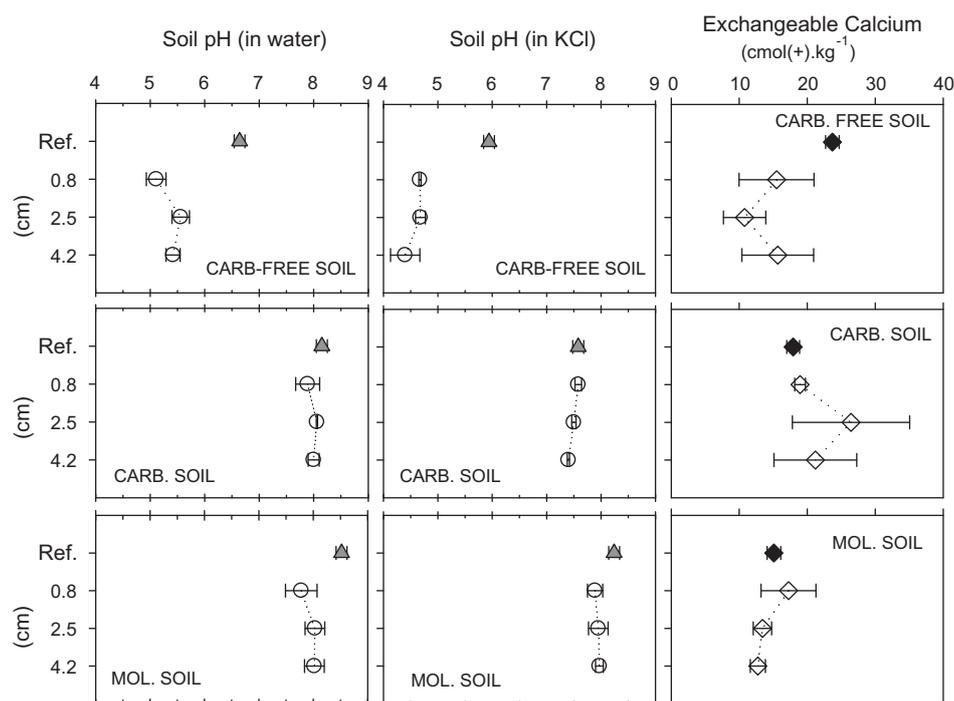
Fig. 5. Variation in pH and exchangeable Ca + Mg content of untreated soil samples and of carbonate free, carbonated and molasse saprolite soils at 0.8 cm, 2.5 cm and 4.2 cm depths in columns after the experiments. Depth (cm) refers to the sample depths in the soil columns.

3.2.2. Exchangeable cations pool

Various changes were detected on soil exchangeable base cations pools following NH_4Cl amendments depending on soil types (Fig. 5 and Table 4). The amount of exchangeable calcium decreased strongly in carbonate-free soils following the nitrogen treatments, with a mean loss of 9.7 cmol(+) kg^{-1} of exchangeable Ca^{2+}. In contrast,

no significant difference was observed for carbonated and $Mol._{Sap}$ soil columns (Fig. 5). Another noticeable consequence of the experiment was the release of exchangeable Mn (mean value of 0.4 ± 0.3 cmol(+) kg^{-1} of Mn in Carb.free soil columns). Mn was not detected in the exchange phase of the untreated soil columns or in the exchange complex of other soil types.

Table 4
Variations of Ca^{2+}, Mg^{2+}, Mn^{2+}, K^+ and Na^+ exchangeable content in untreated soil samples (ref.) and at 0.8, 2.5 and 4.2 cm depths in carbonate free, carbonated and molasse saprolite soil columns after the experiments.

Soil type	Depth cm	Ca^{2+} mmol l^{-1}	Mg^{2+}	Mn^{2+}	K^+	Na^+
Carb.free	Ref.	32.25	0.9	0.0	0.5	0.3
	0.8	15.5 ± 5.5	1.3 ± 0.5	0.5 ± 0.3	0.2 ± 0.0	0.5 ± 0.4
	2.5	10.8 ± 3.1	0.7 ± 0.4	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
	4.2	15.7 ± 5.3	1.2 ± 0.5	0.4 ± 0.3	0.2 ± 0.0	0.4 ± 0.1
	Mean	*14.0 ± 4.8*	*1.1 ± 0.5*	*0.4 ± 0.3*	*0.2 ± 0.0*	*0.4 ± 0.2*
Carb.	Ref.	17.93 ± 1	0.5	0	0.3	0.3
	0.8	18.6 ± 0.8	0.4 ± 0.0	0	0.3 ± 0.0	0.3 ± 0.0
	2.5	25.9 ± 8.4	0.6 ± 0.2	0	0.5 ± 0.1	0.3 ± 0.0
	4.2	20.7 ± 5.9	0.5 ± 0.1	0	0.4 ± 0.1	0.3 ± 0.0
	Mean	*20.6 ± 5.1*	*0.5 ± 0.1*	*0*	*0.4 ± 0.1*	*0.3 ± 0.0*
$Mol._{Sap}$	Ref.	15.1	0.3	0	0.1	0.4
	0.8	17.2 ± 4.0	0.4 ± 0.1	0	0.08 ± 0.01	0.3 ± 0.0
	2.5	13.4 ± 1.3	0.3 ± 0.0	0	0.08 ± 0.01	0.3 ± 0.0
	4.2	12.7 ± 1.1	0.3 ± 0.0	0	0.08 ± 0.01	0.3 ± 0.0
	Mean	*14.5 ± 3.0*	*0.3 ± 0.1*	*0*	*0.08 ± 0.01*	*0.3 ± 0.0*

$n = 3$ for each sample type (except for carbonated soil columns for which six samples, corresponding to two analyses for each subsample, were analyzed).

4. DISCUSSION

4.1. Validation of soil column experiments. Comparison of Leaching rate estimations with the literature and field results

Experimental leaching rates of base cations were calculated for the different soil type and for both amendment rates (Table 5).

A leaching rate (Ca + Mg) of 0.07 mol m^{-2} a^{-1} for a runoff of 80 mm (average drainage at the Montoussé catchment outlet) was calculated in the reference column of carbonated free soils (Table 5). Under a temperate climate, mean leaching rates for base cations in watersheds draining granitoid rocks have been reported to be between 0.0258 and 0.0913 mol m^{-2} a^{-1} for runoff values of 128 and 171 mm a^{-1}, respectively (White and Blum, 1995). The experimental value was within this range.

Carbonate dissolution is mainly controlled by the amount of rainfall and, consequently, by drainage (Drever and Hurcomb, 1986; Kiefer, 1994; Egli and Fitze, 2001; Amiotte-Suchet et al., 2003). Experimental leaching rates for carbonated soils were normalised with a standard value, the world average drainage of 301.55 mm yr^{-1} (Amiotte-Suchet et al., 2003). Normalised experimental leaching rates (Ca + Mg) were 0.41 mol m^{-2} a^{-1} and 0.76 mol m^{-2} a^{-1} in the reference columns of carbonated soils and molasse saprolite, respectively (Table 5). These values are in the range of those reported by Egli and Fitze (2001) of 0.51–0.9 mol m^{-2} a^{-1}. Laboratory experiments on silicate weathering have often been found to over-estimate natural weathering rates by several orders of magnitude (White and Brantley, 2003). Soil columns better reproduce field conditions. Soil column experiments on the effect of acid rain on soil acidification have shown that experimental soil leachates agreed fairly well to field leaching fluxes (Liao et al., 1997). Indeed, the soil columns were only five centimetres high and might not have properly reproduced processes occurring in the whole soil column in the field. But, the carbonate leaching rates obtained during this experiment from the reference columns were similar to literature data. The experimental protocol for the soil columns was thus able to reproduce carbonate leaching behaviour. NH$_4$Cl amendments of the soil columns (whatever the input rate) significantly increased cation leaching by about 15-, 12- and 6-fold, respectively, for carbonate free, carbonated and Molasse saprolite soils.

Experimental data were also compared to field measurements. When normalised to a runoff of 301.55 mm a^{-1}, the (Ca + Mg) leaching rate in the Montoussé stream for the hydrological year 2007 (as estimated from intensive sampling) is 1.33 mol m^{-2} a^{-1} with annual mean concentrations for Ca, Mg, NO$_3$ and HCO$_3$ of 2.73 ± 0.65, 1.24 ± 0.26, 0.7 ± 0.26 and 6.14 ± 0.90 mmol l^{-1}, respectively (Perrin, 2008). The (Ca + Mg) leaching rates obtained during the laboratory experiment on amended columns were more than four times higher (Table 5) than the stream leaching rates, according to the same reference average world drainage. This discrepancy between experimental and field results might be explained because: (i) field values are calculated from the stream data, not from soil column data; (ii) the experimental input of fertiliser was an extreme fertilisation. The mean leaching rate evaluated from the stream survey represents an average of different fertilisation spreading periods, including different forms of fertiliser. Indeed, the farmers in the Montoussé catchment have adapted their agricultural practices recently in order to minimise nitrate leaching to surface waters. It can be assumed that *in situ* field concentrations observed in this study correspond to a minimal concentration range for other non-controlled cultivated areas.

Consequently, we can suppose that the experimental design was able to adequately reproduce natural leaching rates. Nitrification input on the soil column significantly increased base cation leaching. Nevertheless, the evaluated

Table 5
Leaching rates of (Ca + Mg) for the carbonate free, carbonated and molasse saprolite soil columns with or without NH$_4$Cl treatments and corresponding runoff values.

Soil types	(Ca + Mg) leaching rates mol m^{-2} a^{-1} Mean	Runoff mm Mean	Minimum	Maximum
Carb. free				
Reference column	0.07	80		
Treatment 1	0.85 ± 0.17	112	107	117
Treatment 2	1.24 ± 0.25	125	111	135
Treatments average	*1.05 ± 0.21*			
Carb.				
Reference column	0.17 (*0.41*)	127 (*301.55*)		
Treatment 1	1.60 ± 1.14 (*9.10*)	53 (*301.55*)	49	58
Treatment 2	2.48 ± 0.30 (*8.04*)	93 (*301.55*)	68	131
Treatments average	*2.04 ± 2.34*			
Mol. Sap				
Reference column	0.30 (*0.76*)	119 (*301.55*)		
Treatment 1	1.84 ± 0.47 (*10.9*)	51 (*301.55*)	42	60
Treatment 2	1.67 ± 0.13 (*10.1*)	50 (*301.55*)	24	87
Treatments average	*1.76 ± 0.3*			

In italic: for carbonated soils, leaching rates are given for a runoff world average value of 301.55 mm (see Amiotte-Suchet et al., 2003).

increase of the leaching rate after fertiliser spreading on the soil column corresponded to an extreme condition.

4.2. Extent of nitrification in different soils

Nitrification of ammonium inputs to the soil column occurred to different extents in the different soil types. Low nitrification rates were observed in the non-carbonated soil and in the molasse soil columns. Conversely, high nitrification rates were observed in the carbonated soil. Many parameters influence the ammonium nitrification rate in soils, including temperature, soil moisture and structure, pH and ammonium adsorption in soil (Quastel and Scholefield, 1951; Montagnini et al., 1989). Low pH can be a limiting factor of nitrification reactions (e.g. Kyveryga et al., 2004; Kemmitt et al., 2005), which can explain the lower rates observed in the non-carbonated soil. On the other hand, nitrification was also low in the molasse saprolite soils, which indeed exhibited a higher pH. These soils were collected in the deeper layers (1 m depth). The lack of fresh organic matter in the deeper soil layers can lead to a reduction in bacterial activity (Fontaine et al., 2007). Moreover, the dense structure of the saprolite soil may have limited the contact between ammonium and bacteria and thus the nitrification process (Quastel and Scholefield, 1951). The highest rates of nitrification were observed in the carbonated soil, with a high pH. The optimum pH for nitrification has been observed at pH 8.5 (Quastel and Scholefield, 1951). Lower nitrification rates were observed for each soil type during the second experimental treatment when a larger amount of ammonium was added to the soil. An excessive amount of ammonium has been shown to reduce nitrification rates (Shaviv, 1988).

4.3. Ammonium transformations in soils and weathering reactions

Different processes can occur following the input of ammonium on soils as represented in Fig. 6. These will lead to different leaching rates of cations.

(1) One mole of NH_4 brought to the soil column can be exchanged in the soil exchangeable complex and lead to the leaching of 0.5 mol of Ca.
(2) This mole of NH_4 brought to the soil column can also be oxidised to one mole of NO_3 (nitrification

process) leading to the production of two moles of protons, which will be released to the soil solution (Eq. (1)).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad (1)$$
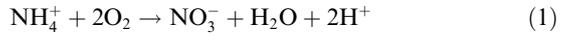
In this case, the protons that are produced can be neutralised in two ways:

(3) either by exchange processes with base cations in the soil exchange complex (Eq. (2))

$$Soil - Ca^{2+} + 2H^+ \Longleftrightarrow Soil - 2H^+ + Ca^{2+} \qquad (2)$$

or through carbonate mineral dissolution if the soil contains any (Fig. 6).

$$2Ca_{(1-x)}Mg_xCO_3 + 2H^+ \Longleftrightarrow 2HCO_3^- + (2-x)Ca^{2+} + xMg \qquad (3)$$
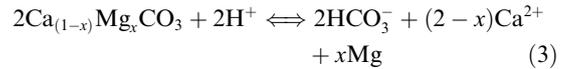
Consequently, the dissolution of carbonate minerals by protons produced by the nitrification of NH_4^+ fertilisers is supposed to involve direct release of cations and alkalinity in the soil solutions following Eq. (4) (see details in Perrin et al., 2008), which is a combination of Eq. (1) and Eq. (3).

$$2Ca_{(1-x)}Mg_xCO_3 + NH_4Cl + 2O_2$$
$$\rightarrow 2(1-x)Ca^{2+} + 2xMg^{2+} + NO_3^- + H_2O + 2HCO_3^- + Cl^- \qquad (4)$$
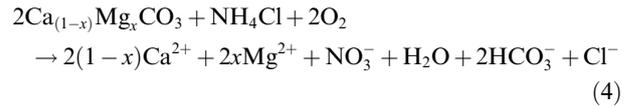
If only this reaction occurs, $(Ca + Mg)/NO_3$ and $(Ca + Mg)/Cl$ ratios in soil solution will be 2:1. Nevertheless, carbonated soils can be considered as an open system in equilibrium with a gas phase of fixed CO_2 pressure (Drever, 1997; Drake, 1983). Thus, depending on the chemical conditions in the soil, different processes can happen in the soil solution:

(i) If the pH of the soil solution is locally reduced (below 6.3), like in microsites where nitrification reaction occurs, HCO_3^- can react with H_2O, leading to H_2CO_3 formation and CO_2 emission. The final equation will be:

$$Ca_{(1-x)}Mg_xCO_3 + NH_4Cl + 2O_2$$
$$\rightarrow (1-x)Ca^{2+} + xMg^{2+} + NO_3^- + CO_2 + 2H_2O + Cl^- \qquad (5)$$
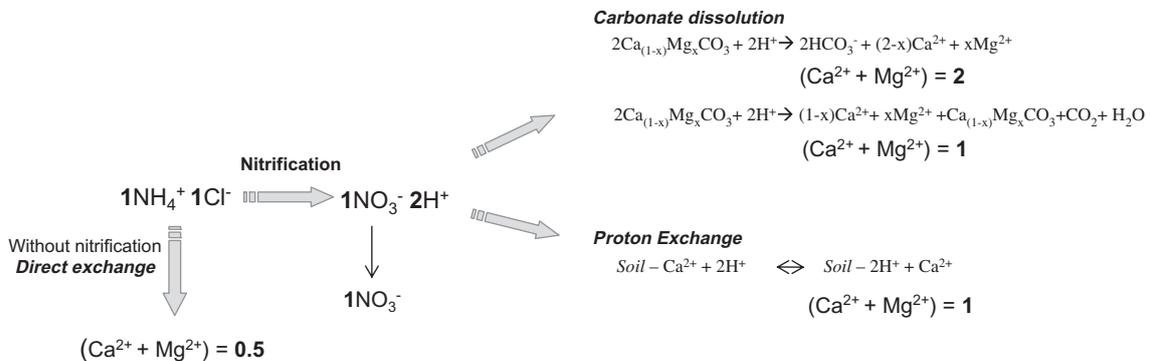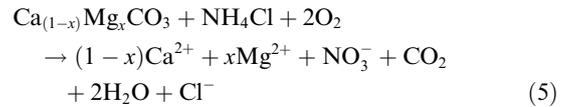
Fig. 6. Schematic representation of the different NH_4Cl behaviors in carbonated soils.

In this case, one mole of calcareous mineral dissolved will lead to one mole of CO_2 emission. In the soil solution, $(Ca + Mg)/NO_3$ and $(Ca + Mg)/Cl$ ratio will be equal to one. One mole of Ca^{2+} in the soil solution corresponds to one mole of emitted CO_2.

(ii) If the soil solution is close to the calcareous mineral saturation index, as might happen deeper in soil profiles or in the river bank, direct secondary precipitation might occur (Eq. (6))

$$2HCO_3^- + (1 - x)Ca^{2+} + xMg^{2+}$$
$$\rightarrow Ca_{(1-x)}Mg_xCO_3 + CO_2 + H_2O \qquad (6)$$
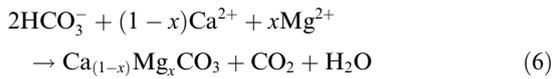
This will lead to direct CO_2 emission, as calcareous mineral was firstly dissolved without CO_2 consumption (Eq. (7))

$$2Ca_{(1-x)}Mg_xCO_3 + NH_4Cl + 2O_2$$
$$\rightarrow Ca_{(1-x)}Mg_xCO_3 + NO_3^- + 2H_2O$$
$$+ CO_2 + (1 - x)Ca^{2+} + xMg^{2+} + Cl^- \qquad (7)$$
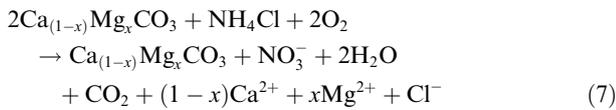
This reaction is more likely to occur in the studied soils with high pH.

Eq. (7) shows that two moles of dissolved calcareous mineral will release one mole of $(Ca + Mg)$ in the soil solution and one mole of CO_2. The ratios $(Ca + Mg)/NO_3$ and $(Ca + Mg)/Cl$ in soil drainage solutions will also be equal to one.

As previously discussed, in this experiment, nitrification was not complete and other processes (such as direct exchange between NH_4 and base cations) could have led to base cation leaching. The study of the correlation coefficients between base cation and nitrate fluxes in the soil leachates can be used as a clue to indicate which dominant process led to base cation release in the soil solution.

4.4. Soil and soil solution modifications as induced by N fertiliser inputs

In carbonate-free soils, during the first treatment, nitrification was partial and Ca leaching was both due to exchange with protons and ammonium. This later became dominant during the second treatment. The base cations to chloride ratio was close to 0.5. Besides, soil pH was significantly decreased during the experiment (mean loss of 1.3 units). The amount of protons on cation exchange capacity (CEC) was significantly increased (23-fold on average for the whole column) indicating that even if the nitrification reaction was not complete in soil columns, CEC was deeply modified. The carbonate-free soil exchange complex was enriched in protons and manganese ions, while depleted in exchangeable base cations (Table 4). The protons produced during the nitrification processes were not neutralised by weathering processes but by cation exchange, leading to soil acidification and exchangeable complex depletion (Stumpe and Vlek, 1991; Poss and Saragoni, 1992; Cahn et al., 1993; Nohrstedt, 2001; Frank and Stuanes, 2003). Manganese ions that were released might have originated from the weathering of mineral or hydroxide concretion (Stumpe and Vlek, 1991; Kogelman and Shape, 2006).

In the molasse soil columns, the nitrification process was also not complete. Regression slopes between $(Ca + Mg)$ and Cl fluxes were between 0.5 and one, indicating that Ca was leached in the soil solution by direct exchange with NH_4, but also following exchange with protons produced during nitrification.

In carbonated soils, the nitrification rate was higher, and almost complete after the first treatment. During the first treatment, the $(Ca + Mg)/Cl$ ratio was close to one and the $(Ca + Mg)/NO_3$ ratio equalled 1.3, which indicated that base cation leaching was mostly due to exchange processes with protons emitted during nitrification, as previously observed by Faurie (1977). Especially during the second treatment, we can suppose that direct dissolution of calcite occurred, because the correlation coefficient between base cations and nitrates was between one and two (1.44). Besides, the $(Ca + Mg)/Cl$ ratio remained close to one and HCO_3 measured in the soil solution was particularly low. Since pH was high (above 8) and soil solution was saturated regarding to calcite (slightly positive saturation indices were calculated with Phreeqc software for the calcareous soil solution during both experiments), direct reprecipitation might have occurred (Eq. (6)), leading to direct CO_2 emissions. Protons produced by nitrification were neutralised with no pH change, as indicated by the comparison of soil pH between the reference and treated columns.

In soils containing carbonates (carbonated and molasse), no significant changes in pH values were detected after the experiment. Because protons were not detected on the exchangeable complex after the experiment and because the amount of exchangeable cations had increased, we may suppose that carbonate minerals weathering might have occurred, either to neutralise protons emitted during nitrification or naturally to replenish the cation exchange complex. In both cases, the released base cations replenished the exchange complex. Weathering reactions buffered proton emissions in the soil solution. Mineral (anorthite and biotite) weathering due to nitrification processes was also evoked by Berthelin et al. (1985) in forest soils and plagioclase (Pierson-Wickmann et al., 2009) in agricultural soil to explain cations leaching after nitrification. Carbonate minerals are much more reactive to acidity than are silicate minerals. This process has a consequence on the carbon cycle, as in this case the carbonate mineral dissolution occurred without natural atmospheric CO_2 consumption and it was supposed to lead to direct CO_2 release (Perrin et al., 2008). However, the quantification of direct CO_2 release could not be determined based on our experiments. Concomitant processes may have occurred, including the exchange of base cations with ammonium and protons released by ammonium oxidation.

4.5. Consequences of nitrogen inputs on soils: implications for cation leaching rates, CO_2 budgets and limits of the approach

The basic reactions for carbonate rock weathering can be expressed by

$$Ca_{(1-x)}Mg_xCO_3 + CO_2 + H_2O$$
$$\leftrightarrow 2HCO_3^- + (1 - x)Ca^{2+} + xMg^{2+} \qquad (8)$$
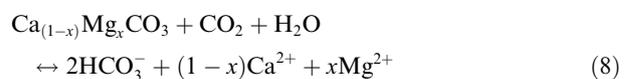
Carbonate rock weathering contributes to the atmospheric CO_2 sink, but only half of the carbon in solution is from atmospheric CO_2 (Eq. (7)). From our study, it can be supposed that carbonate dissolution by protons from NH_4 nitrification modified the CO_2 budget in different ways:

(1) This may have led to direct CO_2 release by titration of HCO_3 in soils (this hypothesis makes the assumption that carbonated soils are open systems in which CO_2 does not concentrate, see details in Perrin et al., 2008).

(2) This may have increased base cation leaching through amplified weathering reactions (without CO_2 consumption), and each additionally released mole of base cations will: (i) either be transferred via the riverine system to the ocean, where it is believed to react with two moles of HCO_3^-, precipitating one mole of $CaCO_3$ and releasing one mole of CO_2 (on the geological time scale of 0.083 Ma according to Berner et al., 1983); (ii) or be neutralised by secondary precipitation of carbonate in soils or river beds, which will directly (on a human time scale) release CO_2 into the atmosphere. Liu and Zhao (2000) estimated that 70% (0.3 GtC yr^{-1}) of the 0.41 GtC yr^{-1} that are potentially consumed on a global scale by carbonate dissolution would be neutralised by rapid secondary precipitation of $CaMgCO_3$ in soils and river beds.

Net CO_2 flux for local situations is difficult to estimate. Measured (Ca + Mg) concentrations in the field soil solutions and in the Montoussé streamwater were on average 10.5 lower than concentrations in the experimental leachates. In the soil column experiments, the average (Ca + Mg) flux from carbonated soils for treatments 1 and 2 was 8.6 mol m^{-2} a^{-1} when considering the world average drainage intensity of 301.55 mm a^{-1} (Table 4). If we divide this value by 10.5, we might consider that the obtained value is a reasonable representation (Ca + Mg) fluxes from carbonated soils in N-fertilised conditions of the Montoussé catchment. If we subtract the pristine flux from this value, which can be considered as the measured (Ca + Mg) flux in the reference (blank) columns of the experiment (i.e. 0.41 mol m^{-2} a^{-1}), the estimated (Ca + Mg) flux that can be attributed to the weathering of carbonate by protons from nitrification reactions equals about 0.45 mol m^{-2} a^{-1} (i.e. 0.86–0.41) (Table 4). This estimated value is supported by a recent study which showed that a significant portion of dissolved inorganic carbon export in riverine waters of small catchments, ranging from 0.43 to 0.86 mol C m^{-2} a^{-1}, could be related to agricultural and urban activities in the Eastern U.S. (Barnes and Raymond, 2009).

On a global scale, if the surface of fertilised areas that drain carbonate substrates (12.35×10^6 km^2 i.e. 12.35×10^{12} m^2, see Perrin et al., 2008) is considered, these results would imply an additional flux of 5.7×10^{12} mol a^{-1} of Ca + Mg. These cations mostly come from carbonate weathering without CO_2 consumption and the rapid release of CO_2 with secondary precipitation of cations is likely to occur on a human scale (Liu and Zhao, 2000).

Nevertheless, these experimental results have to be considered with care since numerous limitations arise from the experimental setup. Although the soil columns were shown to closely reproduce field leaching fluxes, the 5 cm experimental column was unlikely to represent the processes occurring in the whole soil column and in the stream. Moreover, the amount of NH_4 introduced to the soil columns corresponded to extreme fertilisation conditions. On the other hand, sulphuric acid originating other types of fertilisers or from atmospheric inputs might be another source of protons (Amiotte-Suchet et al., 1995), which were not considered here. Finally, the crop influence was not considered in this study. However, the adsorption of nitrates and ammonium by vegetation as well as the base cation export through harvesting would assuredly influence the cation leaching budget (Jaillard et al., 2002; Hinsinger et al., 2003) as well as mineral weathering (Lucas, 2001; Hinsinger et al., 2001). Furthermore, the role of plants on water dynamics should also be considered. Nitrification rates could be lower in nature because of less favourable temperature conditions.

Despite these limits, this study demonstrated that N-fertilisation in carbonated areas assuredly has a significant impact on cation and CO_2 budgets that was previously underestimated in Perrin et al. (2008) and deserves further field investigations.

5. CONCLUSION

This study provides new insights into the consequences of fertiliser inputs on agricultural soils and the implications of fertilisation on cation and carbon budgets.

Nitrification rates depended on the soil type, and were found to be mostly dependant on soil pH and soil structure. The highest nitrification rates were observed in the surface carbonated soil.

In carbonate-free soils, ammonium input on soil induced base cation leaching and a decrease of soil base saturation. No buffering reaction occurred to neutralise protons emitted by the nitrification process. Thus, nitrogen fertiliser inputs lead to soil acidification (an increase in the exchangeable proton pool in soils, and of soil sensitivity to acidic inputs).

In carbonated soils, nitrogen fertiliser inputs led to a tremendous base cation leaching without any decrease of the CEC or observed soil acidification. Then, carbonate mineral weathering was stimulated and this buffered the acidification induced by ammonium nitrification. Increased carbonate mineral weathering occurred following fertiliser inputs. Carbonate weathering reactions, when induced by protons from N fertiliser nitrification, did not always consume CO_2. Acidification induced by the nitrification of nitrogen inputs modified the carbon cycle. Depending on the local chemical conditions, the potential release of CO_2 might be substantial. Indeed, this first experimental approach indicated that if these results are extrapolated to a global scale for the surface of fertilised areas lying on carbonate, carbonated reactions with N fertilisers would imply an additional flux of 5.7×10^{12} mol a^{-1} of Ca + Mg. Thus, the modification of weathering reactions in cultivated catchments and the potential of nitrogen fertilisers to

significantly modify the CO_2 budget deserves much more attention from the research community and should be considered in carbon global cycle modelling.

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