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Investigation of biogeochemical activities in the soil and unsaturated zone of weathered granite

C. LEGOUT[1,*], J. MOLENAT[1], S. LEFEBVRE[2], P. MARMONIER[2] and L. AQUILINA[3]
*[1]IFR CAREN, Sol Agronomie Spatialisation, INRA Rennes, France; [2]IFR CAREN, Ecosystèmes Biodiversités Evolution, CNRS-Univ. Rennes I, France; [3]IFR CAREN, Géosciences Rennes, CNRS-Univ. Rennes I, France; *Author for correspondence: Present address: UMR INRA ENSAR, 65 Rue de Saint Brieuc, CS 84215, 35042 Rennes cedex, France (e-mail: cedric.legout@rennes.inra.fr; phone: +33-2-23-48-54-32; fax: +33-2-23-48-54-30)*

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Abstract. This study, based on field and laboratory work, investigates the biogeochemical activity below the organic top soil horizons, particularly the potential for nitrate removal processes in the deep vadose zone (1–2.5 m depth) of a weathered granite. An experimental site located in the Kerbernez agricultural catchment (Brittany) has been equipped with ceramic cups from 0.5 to 2.5 m depth since November 2001. This arrangement allowed collection of water samples from the soil profile and the upper part of the unsaturated weathered granite. Samples were analysed twice a month for chloride, nitrate and sulphate concentrations over a period of 2.5 years. Laboratory measurements were carried out on three soil horizons and four weathered granite facies sampled in October 2003 for hydrolasic activity, potential nitrification, potential denitrification and batch experiments to study nutrient dynamics. Anion analyses in the field show that the nitrate and chloride concentrations were linearly correlated at each depth. The nitrate/chloride ratio decreased with depth in the upper part of the weathered granite from 4.93 to 2.82. This suggests that nitrate was removed during its vertical transport in the unsaturated zone. The laboratory experiments show that the bacterial activity decreased with depth. However, a significant potential for biogeochemical reactions exists below the organic soil layers. The denitrification rates obtained in the laboratory were significant, up to 800 ng N h^{-1} g^{-1} after about 100 h of incubation for the most reactive weathered granite facies. These rates agree with effective rates usually measured in riparian zones, but they were 50 times higher than those observed in the field. This difference suggests that the denitrification processes occurring in the field were spatially limited to localised anaerobic microsites, where the bacterial activities are controlled by the availability of N and C substrate. Finally, the laboratory measurements lead us to assume that heterotrophic denitrification was clearly the predominant process occurring in the field because of the good correlation between nitrate concentration variation and carbon content ($r = -0.94$). Moreover, the slight increase in sulphate concentrations observed in the field and in the laboratory was insufficient to explain the complete removal of nitrate.

Introduction

Nitrate contamination of surface water has become important in Brittany during the last 30 years. Preventing nitrate pollution by modifying agricultural practices requires improving our understanding of the transport and biogeochemical transformations at the catchment scale.

Most of the studies have investigated the transport and transformation of N and C either in groundwaters or in top soil layers. The mechanisms responsible for nitrate transport from the groundwater to the river are well documented in Brittany (Dia et al. 2000; Molenat et al. 2002; Clément et al. 2003; Martin et al. 2004). Heterotrophic and autotrophic denitrification processes in groundwater have also been quantified in different contexts (Mariotti 1986; Wassenaar 1995; Montgomery et al. 1997; Pauwels et al. 2001). The organic top soil horizons are usually considered as highly biogeochemically reactive. Below this zone, the biogeochemical processes are often neglected. Modelling of the nitrogen cycle is reduced to physical transport mechanisms involving nitrate dilution or dispersion, which consider the nitrate as a conservative constituent. However, biogeochemical processes such as denitrification do not only occur in constantly saturated zones (Parkin 1987; Luo et al. 1999; Hefting et al. 2004). Such processes can have a significant effect on the amount of nitrate reaching the water table during vertical transport of water in the vadose zone (Jarvis and Hatch 1994; Richards and Webster 1999).

Even though the highly bioreactive character of the organic soil horizons is now well documented, few studies have focused on the unsaturated zone below the organic soil horizons (Jarvis and Hatch 1994; Richards and Webster 1999). In the present study, we aim at quantifying the bacterial activity, especially the denitrification, in the unsaturated zone below the first 50 cm of soil. The main characteristics that distinguish deep unsaturated zones from highly reactive top soil horizons are the low carbon content, the slow rate of aeration and the longer residence time of solutes. Although the low availability of organic carbon could limit heterotrophic processes, the other characteristics do not limit potential bacterial reduction processes in the unsaturated zone. In catchments with fractured granite basement, shallow groundwater develops and fluctuates in the weathered zone. In the context studied here, the upper part of the variably saturated weathered granite was the zone of the water table fluctuation, situated close to the organic soil horizons. Any increase of the water content leads to diffusion, allowing the access of nitrate or dissolved carbon to reducing microsites where denitrification can occur (Luo et al. 1999).

The goals of this study were (i) to investigate the extent of the biogeochemically reactive zone along soil-weathered granite profiles and, more precisely, (ii) to characterise the occurrence of denitrifying bacterial activity in a variably saturated weathered granite. These two issues were addressed following both field and laboratory studies. The fieldwork consisted of measuring solute concentrations along a vertical soil-groundwater profile over a period of 2.5 years. The laboratory experiments were performed on soil and weathered granite samples to quantify bacterial activities.

Materials and methods

The Kerbernez catchment

The Kerbernez catchment is located in South Western Brittany (Figure 1a). It is an agricultural catchment with an area of 0.12 km^2. The climate is oceanic with a mean annual precipitation for the last decade of 1167 mm Standard Deviation (SD = 195 mm) and a mean potential evapotranspiration of 616 mm (SD = 71 mm). The mean daily temperatures calculated for the last

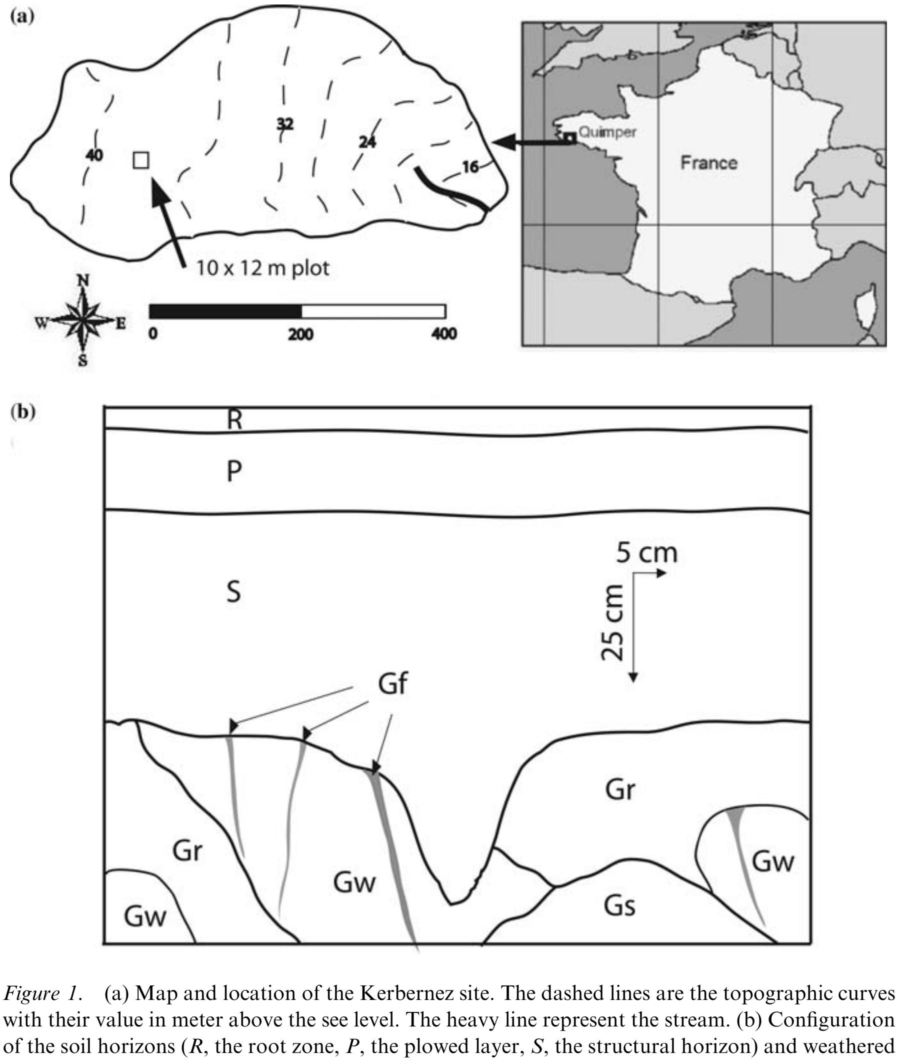

Figure 1. (a) Map and location of the Kerbernez site. The dashed lines are the topographic curves with their value in meter above the see level. The heavy line represent the stream. (b) Configuration of the soil horizons (*R*, the root zone, *P*, the plowed layer, *S*, the structural horizon) and weathered granite facies (*Gr*, red granite with a destroyed structure, *Gw*, white granite with a destroyed structure, *Gf*, red fingering facies and *Gs*, facies with preserved structure) in the trench.

decade are 12.1 °C in the air (SD = 4.6 °C, minimum −4.5 °C, maximum 26.6 °C) and 12.7 °C in the soil at 0.5 m (SD = 3.7 °C, minimum 4.4 °C, maximum 23.7 °C). The bedrock is made up of fissured and fractured granite, overlain by weathered material. The thickness of the weathered granite varies from 1 to more than 20 m. The weathered granite appears between 0.7 and 1.2 m below the soil surface. The soils are mainly sandy loam soils. The groundwater accumulates and fluctuates mainly within the weathered granite, the water table depth ranging from 2 to 8 m below the soil surface through the year.

Water chemistry in the soil and weathered granite

The water chemistry in the soil and the weathered granite were measured along a vertical soil groundwater profile in the Kerbernez catchment from November 2001 to July 2004 (2.5 years). A 10 × 12 m plot was equipped with instruments to sample water in the vadose zone (partially saturated zone) and in the groundwater (totally saturated zone). The plot is located in a plateau area, on grassland pastured since 1996 to April 2003, where maize is now cultivated. Three sections of four ceramic cups (8 cm long, 3 cm diameter) were installed at 0.5, 1.5, 2.0 and 2.5 m depth using a hand auger with a diameter slightly smaller than the diameter of the ceramic cups. So the soil and weathered granite were not disturbed by the ceramic cups installation. These ceramic cups were used to collect water samples in the vadose zone by applying a depression of 0.6 bar during 24 h. Groundwater samples were collected through piezometers at 5, 10 and 20 m depth. The piezometers were composed of PVC tubes with a 1 m thick perforated screen at their extremity, allowing the collection of water from a 1-m-thick groundwater layer. Except for the ceramic cup at 0.5 m, the other ceramic cups and piezometers allowed us to collect water from the weathered granite. The sampling frequency varied between weekly to monthly during the recharge period and the summer, respectively. Physicochemical parameters such as pH, temperature, and electric conductivity were measured during the sampling. The water samples were filtered in the field with a 0.2 μm polytetrafluoroethylene membrane before storing. They were stored less than 24 h in the dark below 4 °C and analysed for chloride, nitrate and sulphate concentrations by ionic chromatography (Dionex DX 100). The total dissolved carbon (TDC) and the dissolved inorganic carbon (DIC) were analysed with a Shimadzu TOC 5050A. The dissolved organic carbon (DOC) was calculated as the difference between TDC and DIC.

Bacterial activity measurement

The measurement of bacterial activity was performed on soil and weathered granite samples in the laboratory. Laboratory experiments were preferred to *in-situ* measurements due to the lack of reliable *in-situ* methods that are easy to use and applicable in a variety of environments. The difference observed between *in-situ* and laboratory measurements may be related as much to spatial variability as laboratory effects (Smith et al. 1996; Well et al. 2003). The sediment samples were collected in October 2003 in a trench – 20 m long and 1.2 m deep – close to the 120 m^2 experimental site (Figure 1b). In this trench, soil samples were collected at various depths:

- at 5 cm below the soil surface, in the Ray-grass roots exploration zone (*R*),
- at 20 cm in the ploughed layer (*P*),
- at 50 cm in the structural horizon (*S*).

The weathered granite exhibited facies with different colours, textures and structures:

- white weathered granite with a destroyed structure (*Gw*),
- red weathered granite with a destroyed structure (*Gr*),
- weathered granite with a preserved structure (*Gs*),
- 1-cm wide and 0.5–1-m long red fingering facies existing in *Gw* (*Gf*).

Samples of each soil and weathered granite facies were collected in this trench along four profiles, and stored in the dark at 4 °C until beginning measurements the next day. Three types of bacterial activity were measured in the laboratory: hydrolasic activity, potential nitrification, potential denitrification and long-term batch experiments to study anion dynamics. All measurements were performed on four replicates of each soil and weathered granite facies. Each sample was analysed for textural properties and total carbon (TC) and total nitrogen (ThermoFinnigan Flash 1112).

Hydrolasic activity

The total esterase activity (Battin 1997) was estimated using fluorescein diacetate (FDA) hydrolysis (Chrzanowski et al. 1984) with fresh sediment. From each sample, three grams of soil received 0.1 ml of FDA and 3 ml of phosphate buffer ($pH = 7.6$), and were then incubated for 10 min in the dark at ambient temperature (Fontvieille et al. 1992; Marmonier et al. 1995). The biological activity was stopped using 3 ml of acetone, which inhibits FDA hydrolysis (Battin 1997). The fluorescein concentration of the filtered supernatant (filter Whatman GF/C) was measured at 490 nm (Uvikon XS spectrophotometer). Incubation time was adjusted to maintain FDA concentration in excess for enzymatic processes. Hydrolysis rate was expressed as μmole of hydrolysed FDA per hour and per gram of dried sediments.

Potential nitrification

The gross rate of nitrification (NT) was estimated using the N-served method (2-chloro-6-[trichloromethyl]-pyridine, known as Nitrapyrin, Sigma Chemical), in which the Nitrapyrin inhibits the function of the mono-oxygenase enzyme and hence inhibits ammonium oxidation (Hall 1984; Dodds and Jones 1987; Prosser 1989; Strauss and Lamberti 2000). Fresh soil from each depth-interval was divided into two 50-g subsamples in the laboratory. Each subsample was placed in a 150 ml flask and 65 ml of groundwater was added. One set of subsamples received a final concentration of 10 mg l^{-1} Nitrapyrin dissolved in dimethyl sulphoxide (DMSO). The other set received only DMSO as a control. The flasks were covered with perforated aluminium foil and incubated in the dark at 25 °C for 24 h on a shaking table (100 rpm). After incubation, extraction of NH_4–N was performed by adding 10 ml of potassium chloride solution (1 N KCl) into each sample and incubating for a second run on a shaking table (100 rpm) for 2 h. The NH_4–N so extracted was analysed by the blue-indophenol method (Solorzano 1969). Gross nitrification rates in both experiments were calculated as the difference in the final NH_4–N concentration measured between the paired Nitrapyrine and control samples (Kemp and Dodds 2002), and expressed per hour and gram of dried soil determined from their water content.

Potential denitrification

Potential of denitrification enzyme activity (DEA) (Smith and Tiedje 1979) was estimated from each sample of fresh soil divided into four sub-samples, which were maintained under anoxic conditions by replacing air with diazote (N_2). The four sub-sample sets were treated as follows:

(i) saturated with distilled water (DEA_A);
(ii) enriched with 10 μg NO_3^-–N g^{-1} fresh sediments (DEA_N);
(iii) enriched with 4 mg glucose g^{-1} fresh sediments (DEA_C);
(iv) enriched with both nitrate and glucose (DEA_{NC}).

The three latter treatments corresponded to optimal conditions for this microbial process (Smith and Tiedje 1979). Acetone-free acetylene, which blocks the denitrification process at the step of nitrous-oxyde (N_2O) production, was added to each flask to bring the flask atmosphere to 10 kPa acetylene and 90 kPa air (%V/V). Slurries were incubated for 8 h at 20 °C. The gas was sampled after 4 and 8 h and analysed by gas chromatography (Chrompack 9001). DEA was calculated based on the difference in N_2O concentrations (measured at 4 and 8 h), being expressed as N_2 produced per hour per gram of dried sediment.

Batch experiments

We investigated the evolution of anion concentrations with time under reducing conditions. Sixty grams of sediment were placed in flasks with 180 ml of groundwater from the 20 m deep piezometer. As with the denitrification protocol, these flasks were maintained in anoxic conditions under a nitrogenous atmosphere. Three millilitres of the solution contained in each flask were sampled with a syringe after 2.5, 24, 48, 144 and 2200 h of incubation. Assuming that the solution was homogeneous after 2.5 h of incubation because of solute diffusion between the groundwater and the water present in the sediment samples, the first sample was taken as the zero point. The 3 ml of solution were analysed by ionic chromatography (Dionex DX 100) for chloride, nitrate, and sulphate concentrations. The chloride was considered as a conservative element. NO_3^- and SO_4^{2-} concentrations were corrected using the variation between chloride concentrations at two sampling times. The mean variations of chloride concentrations so calculated ranged from 1.72% Standard Error (SE = 0.23) to 4.67% (SE = 0.63) over 24 and 2200 h, respectively. To compare the results of these experiments with the denitrification potential measurements, we calculated the mean mass variations of nitrate and sulphate for one hour between two sampling times, i.e. for the periods 2.5–24, 24–48, 48–144 and 144–2200 h.

Results

Water chemistry of soil and weathered granite

Figure 2 presents the nitrate and chloride concentrations available over the 2.5-year period, the data being plotted on NO_3^- Cl^- diagrams for each depth. These diagrams show that both species had highly variable concentrations, especially at shallow depths in the soil (0.5 m) and in the upper part of the weathered granite at 1.5 m. The concentration range tended to decrease with increasing depth: the nitrate concentration varied up to 400 mg l^{-1} at 0.5 m depth in the soil and only 100 mg l^{-1} at 2.5 m depth in the weathered granite. Moreover, the nitrate and chloride concentrations were linearly related at any given depth (Figure 2). The slope of the regression line (a), which represents the nitrate over chloride ratio, decreased with depth in the unsaturated weathered granite. This nitrate/chloride ratio characteristic of each depth (a) decreased fourfold between the upper part of the weathered granite ($a_{1.5m} = 4.93$) and the permanently saturated zone 20 m below the soil surface ($a_{20m} = 1.07$). We observed a large decrease of this ratio, which fell by a factor of 2.6 between 1.5 and 10 m below the soil surface in the zone of water table fluctuation ($a_{10m} = 1.92$). Between 1.5 and 2.5 m, the decrease in nitrate concentration was correlated with a significant increase in the mean concentration of sulphate from 6.25 to 13.63 mg l^{-1} (Figure 3). An increase of the sulphate concentration was also observed deeper in the groundwater, with median values for the sulphate concentrations of 17.52 and 21.05 mg l^{-1} in the piezometers at 5 and 10 m, respectively.

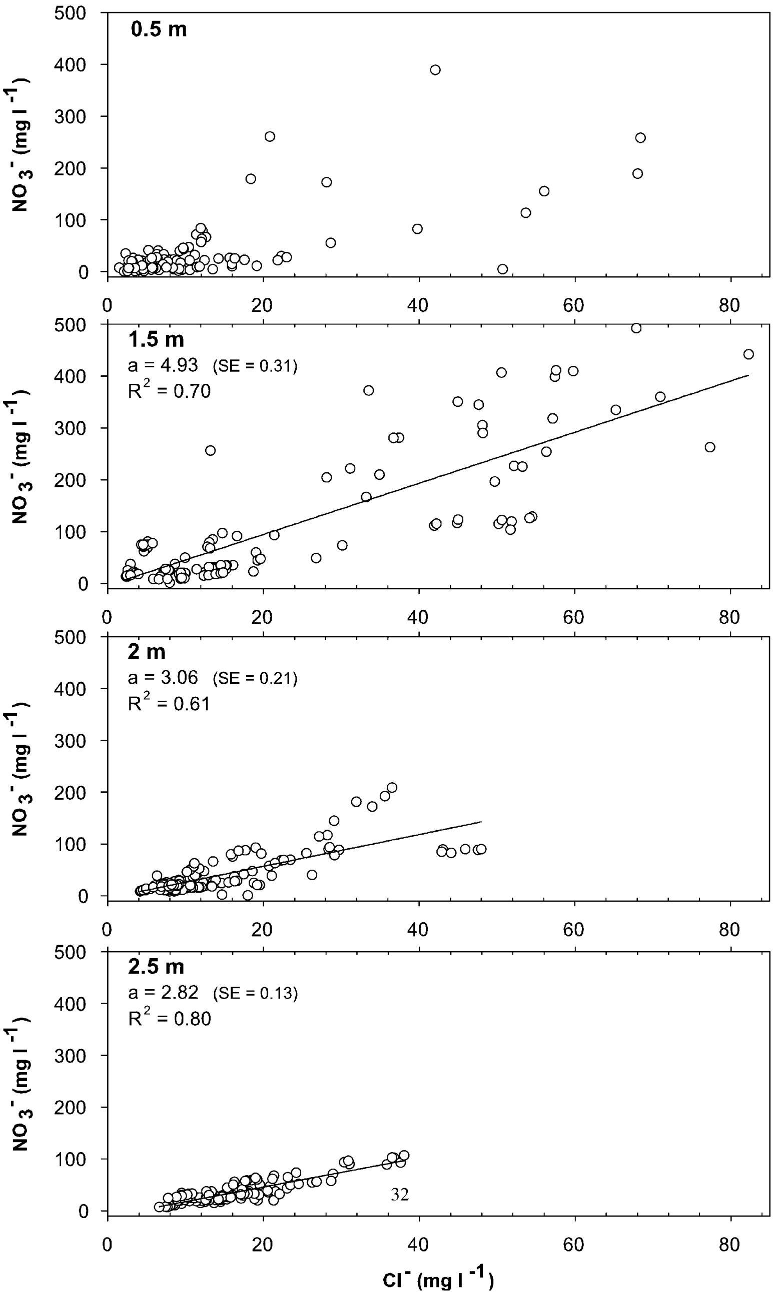

Figure 2. Diagrams of nitrate and chloride concentrations at 0.5 m in the soil and at various depths in the upper part of the variably saturated weathered granite. *a* corresponds to the slope of the regression line, SE is the associated standard error and R^2 the coefficient of determination.

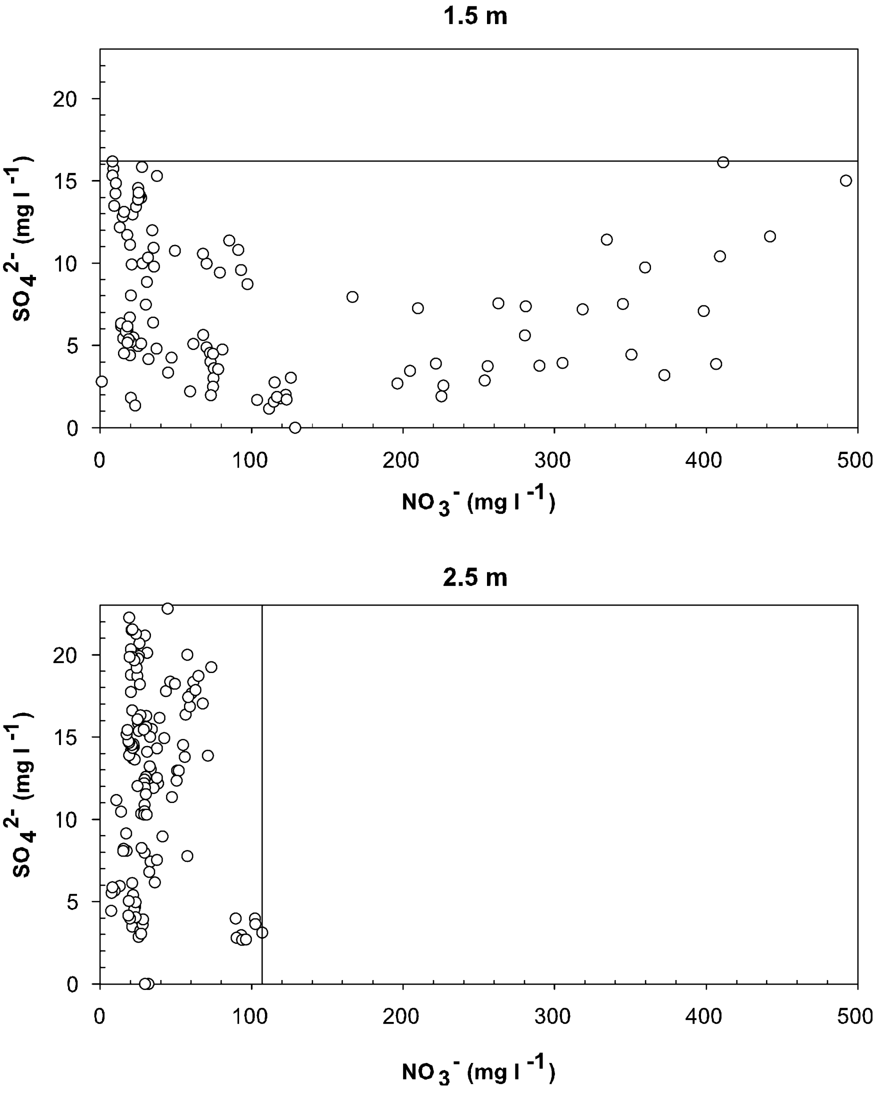

Figure 3. Diagrams of sulphate and nitrate concentrations in the upper part of the weathered granite. The horizontal and vertical lines are upper limits for the sulphate (top) and nitrate concentrations (bottom).

Bacterial activities

The hydrolasic activity decreased with depth. In the soil horizons, this activity decreased twofold between the *R* and *S* horizons (Figure 4a). The hydrolasic activity decreased again in the weathered granite, with strong heterogeneity between material types (Figure 5a). The *Gr* and *Gf* red weathered granite facies had mean bacterial activities significantly higher at $p < 0.05$ (Student-Newman-Keuls Method) than the *Gw* and *Gs* facies.

Similar patterns were observed for potential nitrification in the soil, which decreased with depth (Figure 4b). This potential was three times higher in the *R* horizon than in the *S* horizon. The calculated nitrification potentials were significantly higher in the weathered granite (Figure 5b) than in the soil *S* horizon (Figure 4b). Figure 5b shows that the potential nitrification was significantly higher in the *Gf* red fingering facies than in the other weathered granite facies.

The potential denitrification was measured in the upper soil horizons, *R* and *P*, but levels were below the sensitivity of the method in the *S* horizons and in

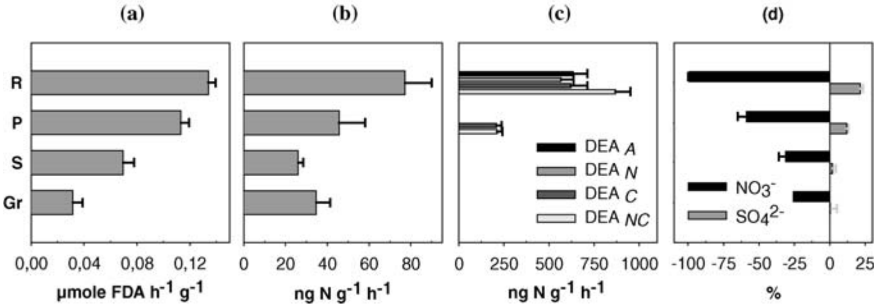

Figure 4. Bacterial activities along the soil-weathered granite profile: (a) hydrolasic activity, (b) potential nitrification, (c) potential denitrification and (d) anion concentration variations calculated from the long term batch experiments during the first 144 h of incubation. The values given for *R*, the root zone, *P*, the plowed layer, *S*, the structural horizon and *Gr*, the red coloured weathered granite facies, correspond to the mean of four replicates. The bars indicate standard errors.

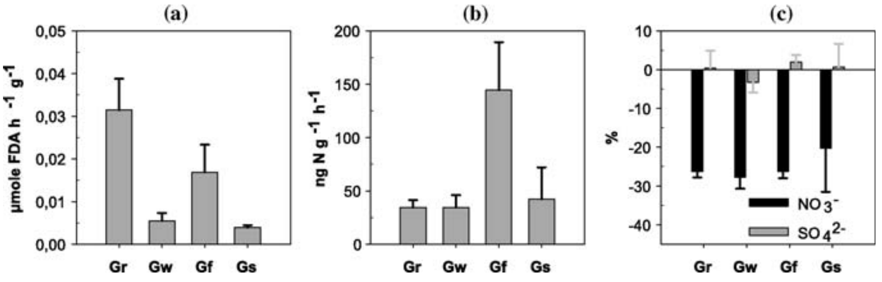

Figure 5. Bacterial activities in the weathered granite: (a) hydrolasic activity, (b) potential nitrification, and (c) anion concentration variations calculated from the long term batch experiments during the first 144 h of incubation. The values given for each weathered granite facies (*Gr*, red granite with a destroyed structure, *Gw*, white granite with a destroyed structure, *Gf*, red fingering facies and *Gs*, facies with preserved structure) correspond to the mean of four replicates. The bars indicate standard errors.

all weathered granite facies (Figure 4c). In the *R* horizon, no significant differences at $p < 0.05$ (Kruskal–Wallis Method) was observed between the DEA_A, DEA_N, DEA_C and DEA_{NC} estimations. At this layer, both carbon and nitrogen contents were high enough to support denitrification. In contrast, the potential denitrification for the *P* horizon was not measurable with DEA_A and DEA_N estimations, but reached 210 ng N g $\mathrm{sediment}^{-1}$ h^{-1} when carbon was added. Biodegradable carbon was the limiting factor for denitrification in this horizon.

Nitrate and sulphate concentrations measured during the batch experiments are displayed in Figure 6. The anion concentrations were higher and much more variable in the upper soil horizons, *R* and *P*, than in the weathered granite samples. After 2200 h, the nitrates were removed from almost all the samples (Figure 6a). By contrast, the sulphate concentrations measured after 2200 h show distinct differences from one horizon to the other and between samples of the same horizon. The sulphate concentration variability between horizons and between samples was much higher at the end of the experiment than at the beginning (Figure 6b).

The evolution of nitrate concentration with time followed the same sigmoidal curve for most of the samples (Figure 6a). The first part of the curve correspond to a slow decrease of the concentrations followed by a more rapid decrease. The first part was very short, less than 24 h for the soil horizons *R* and *P*, and even non-existent for some of them, while it lasted for the first 48 h in the weathered granite samples. The rate of nitrate concentration decrease was maximal during the 24–48 h period for the *R* and *P* soil horizons. In the *Gf* and *Gr* weathered granite facies, the maximum rates of nitrate concentration decrease were rather slower and observed within the 48–144 h period. During the first 144 h, the variations ranged from −99.2% in *R* (Figure 4d) to approximately −25% in the weathered granite facies (Figure 5c). This showed that the rate of nitrate concentration variation decreased with increasing depth. An interesting point is that the mean percentage of the nitrate level variations calculated for the 3–144 h period were similar for the *Gr*, *Gw* and *Gf* facies. These three facies had similar textural and structural physical properties, in contrast with the *Gs* unit close to the granite (Figure 5c).

The sulphate concentration evolutions with time show different steps corresponding to three types of dynamic behaviour. During the first 48 h, 2 groups of samples could be distinguished, one showing a slight decrease and the other a slight increase of the sulphate concentration (Figure 6b). Although the standard errors were large because of the high variability between the four sets of samples, the concentrations tended to increase in the *R* and *P* horizons, whereas they tended to decrease in the weathered granite facies. During the second period, from 48 to 144 h, the sulphate concentrations increased for all the samples. The highest increases were +10.78% (SE = 0.73) for the *R* and +6.19% (SE = 0.61) for the *P* soil horizons, whereas these variations were only 3.11% (SE = 0.42) for the whole set of weathered granite facies. During the last

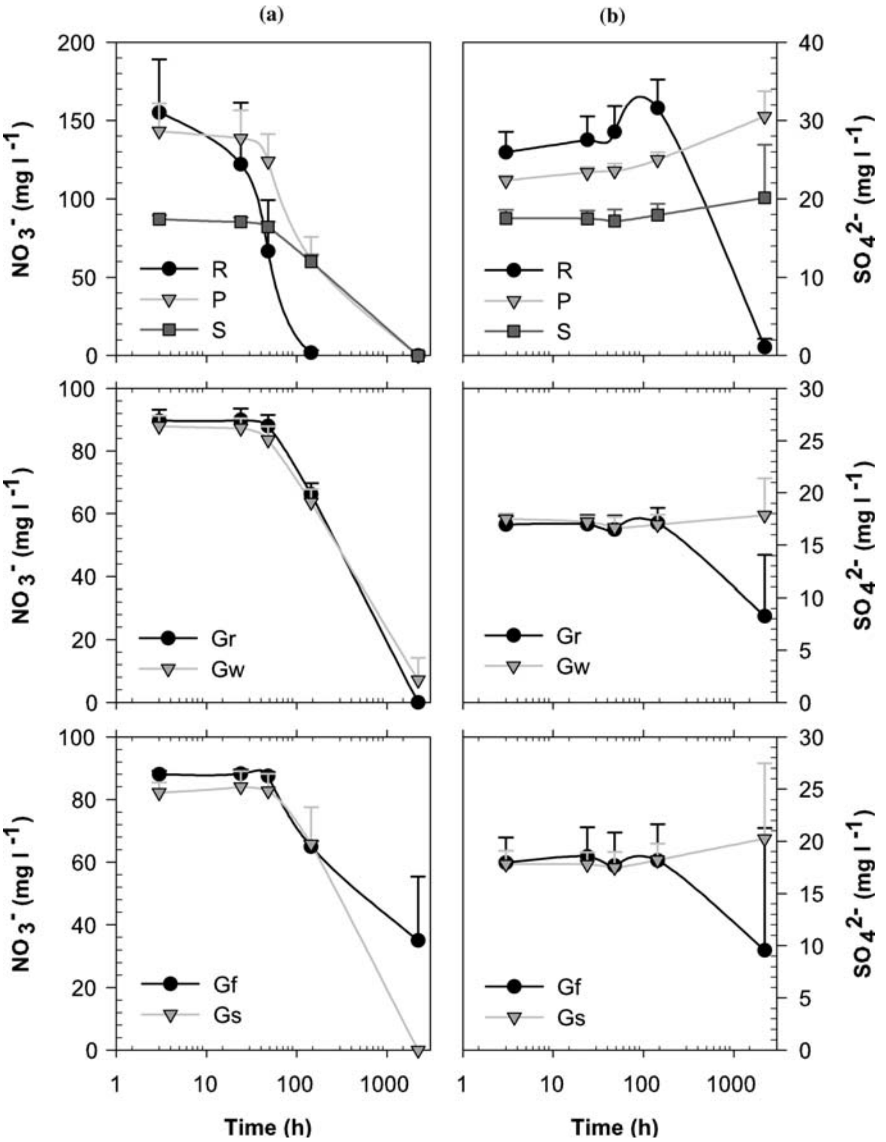

Figure 6. Batch experiments: temporal variations of (a) nitrate and (b) sulphate concentrations in sediment samples under anaerobic conditions. The values given for each soil horizon or weathered granite unit correspond to the mean of the four profiles. The bars indicate standard errors.

period (144–2200 h), two groups of samples could be distinguished, characterised by either an increase or a partial to complete removal of sulphate. The standard errors calculated for the last sampling time, i.e. 2200 h, were very high, implying a considerable variability of the sulphate concentrations. From the beginning of the incubation to 144 h, the hourly variations of sulphate concentrations were very small in comparison with those observed for the nitrates, which were maximal during this period.

Discussion

Does denitrification occur in weathered granite?

The nitrate and chloride concentrations observed in the field displayed two striking characteristics. (1) Nitrate and chloride concentrations showed a positive and linear correlation for any depth in the fluctuating water table layer from 1.5 m down to 10 m. (2) The positive slope of the relationship tended to decrease with increasing depth (Figure 2). The deepest sampled point in the groundwater yielded the lowest ratio ($a_{20\mathrm{m}} = 1.07$). Thus, even though concentrations were highly variable in time and space through the year, especially in the top layer, large nitrate concentrations corresponded nearly always to high chloride concentrations, and inversely low nitrate concentrations were observed along with low chloride concentrations. Moreover, the relationship became more markedly linear as the solutes moved downward to the groundwater ($R^2 = 0.80$ at 2.5 m depth).

The linearity of the relationship observed in the top soil layer could be explained in terms of the nitrate and chloride sources in the Kerbernez agricultural catchment. For both of these anionic solute species, the sources are located in the top soil layer (0–50 cm). Two major sources of nitrate coexist, (i) nitrification resulting from the ammonification of organic matter, and (ii) the oxidation of N-mineral fertiliser spread onto the soil. The chloride can have two origins, represented by rainfall and the agricultural KCl fertiliser (Martin et al. submitted to Chemical Geology). Agricultural fertilisers could be considered as the main source of nitrate and chloride, particularly due to their high levels of anion concentration (Figure 2). In fact, the linearity of the relationship over a wide range of nitrate and chloride concentrations at 50 cm and even 1.5 m depth was due to a mixing between the agricultural source providing high levels of soluble anions and rainfall with low anion concentrations.

We can offer two hypotheses to explain the decreasing slope of the linear relationship between nitrate and chloride concentration with increasing depth. In the first hypothesis, we propose that solutes move downward in the soil in a conservative way and that the decrease of slope with depth reflects variations in the ratio of nitrate to chloride of the source in the top soil layer. A downward decrease of slope implies that the ratio at the soil surface has been increasing with time. Data on the solute velocities in the unsaturated zone were available from tracer experiments undertaken recently on the 10 × 12 m site. Variations in the top soil nitrate/chloride ratio with time seemed unlikely considering that the solute velocity from the soil surface down to 2.5 m depth was around 2.5 m per year on average and that the field observations were carried out over more than 2 years. Indeed, any variation of this source-term ratio would have migrated to greater depth in the soil within the field observation time. However, since the nitrate/chloride ratio remained almost constant in the top layer over the entire field observation period, this same ratio should have been observed deeper within the observation period if the solutes had moved in a conservative way. As further evidence against this hypothesis, we compared the solute concentrations at 1.5 and 2.5 m depth. It took about 150 days (Δt) for solutes to be transported from 1.5 to 2.5 m. Consequently, water collected at 2.5 m depth from May 2002 to May 2004 corresponded to water flowing at 1.5 m depth 150 days earlier, from November 2001 to November 2003. When considering only water concentrations for these two periods and two depths, the nitrate/chloride ratios were 4.72 and 2.67 at 1.5 and 2.5 m, respectively. This shows clearly that the nitrate/chloride ratio varied as solutes moved downward with the water, and that the assumption of a conservative transport for nitrate is not valid.

The second hypothesis to explain the decrease of the NO_3^-/Cl^- slope with depth is to consider that the nitrate transport is not conservative. The main processes causing a decrease of nitrate concentration in soils are plant uptake, immobilisation by microbes, dissimilatory reduction and denitrification. Plant uptake is unlikely to occur for the deepest layers since the depth of grassland roots at the experimental site did not exceed 1 m. Immobilisation by microbes and dissimilatory nitrate reduction to ammonium (DNRA) would conserve the total N amount in the system. The total N amount was not conserved in our system since it decreased with depth (Table 2). Furthermore, DNRA occurrence would imply an increase of $N–NH_4^+$ concentrations with depth. The few data available about $N–NH_4^+$ in water samples collected from ceramic cups in soil between 1.5 and 2.5 m depths show that these concentrations never exceeded 0.3 mg l^{-1} $N–NH_4^+$. A slight increase of the $N–NH_4^+$ concentrations was observed with depth but, these elevations were 100 times smaller than the $N–NO_3^-$ concentration levels or even than the $N–NO_3^-$ decrease between 1.5 m and 2.5 m. $N–NH_4^+$ produced by DNRA could have been re-oxidised latter by nitrification process to $N–NO_3^-$. In this case, the net flux rate of DNRA could not account for nitrate concentration decrease with depth. By contrast, we consider denitrification is likely to occur based on field observations and the results of laboratory experiments. Indeed, the laboratory experiments showed that denitrification could potentially occur in the soil as well as in the weathered granite. The batch experiments show that a complete removal of nitrate was obtained in almost all of the weathered granite samples after 2200 h (Figure 6a). The nitrate removal in the *S* horizon and weathered granite facies occurred at slower kinetics than in the *R* and *P* highly reactive soil horizons. These results were in accordance with the absence of denitrification in the potential denitrification experiment during the first 8 h of incubation in the lower horizons (Figure 4c). In the batch experiments, ideal conditions existed for denitrification because the samples were completely saturated with groundwater under anaerobic conditions. Hence, there were no factors limiting denitrification, which cannot be the case in the field. The field concentrations presented in Figure 2 show that the removal of nitrate was never complete *in situ*. The maximum observed decrease in nitrate concentration between 1.5 and 2 m depths could be estimated at 34%, while it reached only 6.1% between 2 and 2.5 m. This implies that not all the nitrate was removed *in situ*. One reason is that denitrification in the field does not take place throughout the soil or weathered granite volume but in very small local sites as proposed by Parkin (1987) and Luo et al. (1999). These sites were permanently saturated and did not contribute to water flow. Their connection and exchanges with soil aerobic zones were controlled by diffusion, which is a very slow process. The capacity of soil, or weathered granite, to denitrify was then limited by these slow exchanges.

In the hypothesis of denitrification, the rates of degradation in the field and in the laboratory experiments have been calculated and compared. The nitrate concentrations in the soil and weathered granite solution collected from the ceramic cups were high, meaning that the nitrate supply would not be a limiting factor to denitrification, leading to no relationship between nitrate concentration and rate of denitrification. Nevertheless, a zero order kinetics would have conserved the same slope of the NO_3^-/Cl^- relationship with depth. Since this slope decreased with depth, kinetics in accordance with our observations was a first order one. The reason why a one order kinetics fitted the observations much better than a zero order one could be that even if nitrate concentrations were high, the microsites concentrations were lower than the denitrifying enzymes saturation because of limited diffusion. In that case, the denitrification fluxes were proportional to the nitrate concentration, according to the following equation:

$$\frac{\mathrm{d}[\mathrm{NO}_3^-]}{\mathrm{dt}} = -k[\mathrm{NO}_3^-]. \tag{1}$$

The field rate of nitrate removal, k, between two depths have then been calculated from the corresponding difference of the nitrate concentrations and from the travel time, Δt, according to:

$$([\mathrm{NO}_3^-]_{2.5\mathrm{m}} - [\mathrm{NO}_3^-]_{1.5\mathrm{m}}) = -k\Delta \mathrm{t}[\mathrm{NO}_3^-]_{1.5\mathrm{m}}. \tag{2}$$

Since there were linear relationships between nitrate and chloride concentrations at 1.5 and 2.5 m depth, we had:

$$\frac{[\mathrm{NO}_3^-]_{2.5\mathrm{m}}}{[\mathrm{NO}_3^-]_{1.5\mathrm{m}}} = \frac{a_{2.5\mathrm{m}}[\mathrm{Cl}^-]_{2.5\mathrm{m}}}{a_{1.5\mathrm{m}}[\mathrm{Cl}^-]_{1.5\mathrm{m}}} \tag{3}$$

where a was the slope of the regression line. Assuming chloride is a conservative element, $[\mathrm{Cl}^-]_{2.5\mathrm{m}} = [\mathrm{Cl}^-]_{1.5\mathrm{m}}$ and Eq. (2) became:

$$k = \frac{1}{\Delta t} - \left(1 - \frac{a_{2.5\mathrm{m}}}{a_{1.5\mathrm{m}}}\right). \tag{4}$$

Using Eq. (4), the field rate of degradation in the weathered granite, k, was estimated at 10^{-4} h^{-1}. Applying the same type of degradation kinetics to the nitrate concentration decrease obtained in the laboratory, k was around 50 10^{-4} h^{-1}. These results supported the statements of Bidois (1999), who reported differences of two orders of magnitude between potential denitrification measured in the first 20 cm of soils and the effective denitrification rate.

In the laboratory experiments and for the weathered granite samples, we obtained maximum rates of nitrate decrease of 800 ng h^{-1} g^{-1} after about a 100 h of incubation. Bidois (1999) reported maximum but effective denitrification rates of 1000 ng h^{-1} g^{-1} in riparian zones. Consequently, the laboratory results on weathered granite samples were of the same order of magnitude as denitrification rates observed *in-situ* for highly reactive riparian zones. Since the denitrification rates observed *in-situ* were 50 times smaller than those measured in the laboratory, we infer that denitrification occurred in the weathered granite but at rates much slower than in riparian zones (Bidois 1999) or in groundwater (Korom 1992). These results show that limitations exist *in-situ* to this bacterial activity, arising mainly from limitations in the amount of substrate available to be reduced (Gordon et al. 1986).

Type of denitrification

So far, we have presented evidence that denitrification can be effective in the soil and weathered granite. In the following, we discuss what type of denitrification could be involved. Denitrification may be achieved through a combination of heterotrophic and autotrophic denitrification processes (Steinheimer and Scoggin 1998). Such mechanisms occur in anaerobic environments, corresponding to dissimilatory reduction processes that make energy available to the cell for growth and maintenance (Korom 1992).

In the laboratory experiments, we observed two groups of samples that showed either an increase or a decrease of the sulphate concentration from 144 up to 2200 h of incubation. In one group of samples, less and less nitrate became available as the incubation progressed to ensure electron acceptance, so the sulphate was reduced instead of the nitrate. Sulphate reduction is likely to occur when the redox potential is close to -200 mV. Since we never measured values below -35 mV, sulphate reduction would not appear to be strongly expressed in the field. In the other samples, the continuous increase of the sulphate concentration suggests that the oxidation of organic or mineral phases containing H and S took place during the whole incubation period. Since small increases were observed in the sulphate concentration both in laboratory experiments (particularly from 48 to 144 h) (Figure 6b), as well as in the field (Figure 3), the denitrification process could be autotrophic. The sulphate increase could also be ascribed to biological and abiological oxidation or decreases in rates of use of sulphate as an electron acceptor for respiration.

Assuming that the increase of sulphate concentrations derives only from denitrification by oxidation of ferrous disulphide (pyrite) according to:

$$5FeS_2 + 14NO_3^- + 4H^+ = 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O, \tag{5}$$

leads to calculate the maximum autotrophic denitrification rate. The ratio of variations of sulphate concentration ($\Delta[SO_4{}^{2-}]$) and nitrate concentration ($\Delta[NO_3{}^-]$) following reaction (5) is 1.1. For the laboratory experiments, this means that the observed decrease in nitrate concentration of 90 mg l^{-1} related to autotrophic denitrification would lead to an increase of 100 mg l^{-1} for the sulphate concentration. In fact, we observed maximum variations in sulphate that were five times smaller than those observed in nitrate, i.e. $\Delta[SO_4{}^{2-}]/\Delta[NO_3{}^-] = 0.2$. In the field, the maximum value of $\Delta[SO_4{}^{2-}]/\Delta[NO_3{}^-]$ was 0.05. Taking account of the stoechiometric coefficient of the reaction, autotrophic denitrification could not explain all of the nitrate concentration decrease, even in overestimating autotrophic denitrification rate.

Heterotrophic denitrification was clearly the main process occurring in the weathered granite. The role of heterotrophic bacteria in nitrate removal was supported by the observed stimulations of the short-term denitrification by biodegradable carbon (DEA_C and DEA_{NC}) in the ploughed layer *P* (Figure 4c). Denitrification could occur by the oxidation of organic matter according to the following reaction:

$$5CH_2O + 4NO_3^- + 4H^+ = 2N_2 + 5CO_2 + 7H_2O, \tag{6}$$

Since the stoechiometry of denitrification is approximately 1 N:1 C for heterotrophic bacteria, this process can take place even in low-carbon zones (Steinheimer and Scoggin 1998). The upper part of the weathered granite had globally lower carbon than the soil horizons (Tables 1 and 2). However the total carbon (TC) content measured in the *Gf* or *Gr* facies around 2–3 g kg^{-1} were not negligible and even close to common values observed in the bottom soil layers as reported by Cannavo et al. (2002), allowing heterotrophic denitrification to occur. The DOC concentrations were also at high level in the weathered granite up to 19 mg l^{-1} at 2.5 m. A reason for such high levels could be that the upper part of the weathered granite, which is close to the highly biogeochemically reactive soil horizons, is a transition zone characterised by (i) DOC inflow from the overlying soil horizons *S* and *P* with a high TC supply

Table 1. Dissolved organic and inorganic carbon (DOC and DIC) in water samples collected from ceramic cups.

C (mg l^{-1})	0.5 m (n = 21)	1.5 m (n = 27)	2 m (n = 30)	2.5 m (n = 18)
DIC	33.98 (*6.67*)	23.41 (*2.59*)	32.50 (*4.80*)	16.95 (*1.91*)
DOC	26.55 (*1.52*)	11.81 (*1.55*)	12.61 (*2.15*)	19.51 (*2.44*)

The values are mean concentrations of the *n* water samples collected from ceramic cups from December 2001 to May 2002. Values in parentheses are standard errors.

Table 2. Total carbon and nitrogen in soil and weathered granite samples.

	R	*P*	*S*	*Gw*	*Gr*	*Gf*	*Gs*
N (g kg^{-1})	3.52 (*0.18*)	2.21 (*0.06*)	1.08 (*0.09*)	0.03 (*0.03*)	0.17 (*0.06*)	0.28 (*0.05*)	0.00 (*0.00*)
C (g kg^{-1})	38.26 (*2.40*)	22.54 (*0.55*)	11.67 (*1.21*)	1.16 (*0.15*)	2.31 (*0.46*)	3.32 (*0.55*)	0.84 (*0.03*)

The values are mean contents calculated with $n = 4$; values in parentheses are standard errors.

(Table 2) and (ii) the presence of well developed heterotrophic bacteria communities.

Factors controlling denitrification in the field

Results from the soil batch experiments performed in the laboratory show a decrease of the bacterial activity with depth (Figure 4). This decrease is in accordance with previous studies (Dodds et al. 1996; Cannavo et al. 2002). Results also show a potential for biogeochemical reactions (Figure 5a) and nitrate reduction (Figure 6a) in the weathered granite, and that variations of this potential depends more or less on the facies. Nevertheless, effective denitrification rates in the field were 50 times slower than potential rates measured in the laboratory or effective rates reported from riparian zones (Bidois 1999). Two main reasons explain the limited decrease in nitrate concentrations observed in the field between 1.5 and 2.5 m below the soil surface.

The first explanation is the coexistence of nitrification and denitrification processes in the field. Parameters such as total Carbon or total Nitrogen were significantly correlated (at $p < 0.05$) with hydrolasic activity, as well as with parameters describing the kinetics of nitrate concentration decrease with incubation time (Table 3). These two parameters may account for (i) the decrease of the global bacterial activity in the soil (Figure 4) and (ii) the spatial variability of biogeochemical activity between the weathered granite facies, i.e. bacterial activities slightly higher in *Gr* and *Gf* than in *Gw* and *Gs* (Figure 5). The variability of the bacterial activity between the weathered granite facies, was also related to the water content in the field (Table 3) and to the clay content of each facies (9.7% for *Gf*, 6.9% for *Gr*, 4.3% for *Gw* and 1.8% for *Gs*). The higher the clay content, the more intense was the bacterial activity. Under the effect of illuviation processes, facies such as *Gr* or *Gf* would correspond to zones of transport of clay particles. If water is preferentially transferred towards such facies, dissolved Carbon and Nitrogen can be brought in from the upper soil horizons. This could explain why *Gr* and *Gf* were more potentially reactive in terms of nitrate removal, because of the higher global amount of substrate N and C. Nevertheless, if water flows preferentially towards the fingering zones, dissolved oxygen could also be supplied from above, thus contributing to the aerobic nitrification processes. In this way, the effective lowering of nitrate concentrations in the field could also

Table 3. Pearson correlation matrix calculated for the whole soil and weathered granite facies and replicates.

%chg. NO_3^- 3–144 h	%chg. NO_3^- 48–144 h	%chg. NO_3^- 144–2200 h	%chg. SO_4^{2-} 3–144 h	%chg. SO_4^{2-} 48–144 h	%chg. SO_4^{2-} 144–2200 h	FDA	Nitrification	N	C
0.99									
0.12	0.11								
− 0.81	− 0.82	− 0.24							
− 0.89	− 0.89	− 0.21	− 0.79						
0.34	0.37	0.14	− 0.21	− 0.35					
− 0.84	− 0.82	− 0.33	− 0.82	0.80	− 0.14				
− 0.10	− 0.13	0.45	0.14	0.10	0.14	− 0.05			
− 0.94	− 0.93	− 0.24	0.85	0.84	− 0.26	0.94	0.07		
− 0.94	− 0.94	− 0.25	0.85	0.84	− 0.27	0.93	0.07	1.00	
− 0.82	− 0.83	− 0.09	0.74	0.79	− 0.34	0.77	0.29	0.87	0.88

Values in bold characters are correlations with $p < 0.05$. The abbreviation % chg. means the percentage of change in concentration between two sampling times.

be offset by the local production of nitrate through nitrification processes, whose occurrence was clearly demonstrated by the laboratory experiments (Figure 5b).

The temperature could be the factor explaining the difference between field and laboratory denitrification rates. The temperatures measured in the soil by the weather station (average 12.7 °C, minimum 4.4 °C, maximum 23.7 °C) and on the groundwater by direct measurements in the piezometers (average 11.4 °C, minimum 11.1 °C, maximum 11.9 °C) were variable in time and always colder than in the laboratory experiments (20 °C). However, the field temperature range did not prevent denitrification according to other studies reporting denitrification occurrence below 5 °C (Knowles 1982). Moreover, the effect of temperature exists also in riparian zones and yet effective denitrification rates are higher than those measured in our vadose zone.

The water content could be the major factor controlling the denitrification rate. The vadose zone in the field is characterised by a high temporal variability of the water content. This characteristic distinguishes our field vadose observations from the laboratory experiments and the riparian zones. Denitrification must be heterogeneous in the vadose zone and must occur at the aggregate or mineral scale (Parkin 1987). So only small microsites with ideal redoxic conditions, at least with lower redox than those measured at a larger scale in water sampled by the ceramic cups, must be biogeochemically active within the more active facies (*Gr* and *Gf*). Denitrification could be limited by the bioavailability of the N and C substrates, since this was controlled by the increase in water content that allowed diffusion processes. Compared with the laboratory experiments, where the soil or weathered granite samples were saturated with water, denitrification in the field was limited by the low spatial and temporal availability of N and C (Luo et al. 1999), rather than by the slow kinetics.

During periods with low water content, the reducing microsites were only active in facies with sufficient water content. Thus, the diffusion of NO_3^- and soluble C supplied enough substrate for bacterial activity. On the other hand, when the soil became saturated, the convective movements of solutes were too rapid so that NO_3^- removal could not be completed.

Conclusion

The aim of this study was to investigate the potential for biogeochemical reactions, especially denitrification, in the unsaturated zone below the organic soil horizons.

Data from the laboratory experiments show that the bacterial activity decreased with depth and, in particular, there was a noticeable potential for bacterial activity and biogeochemical reactions in the unsaturated weathered granite below the soil organic horizons. The bacterial activity in the weathered granite was highly variable between various facies exhibiting different characteristic structures and textures. These facies yielded distinct bacterial activities, with rates of nitrate removal correlated to carbon content, suggesting the occurrence of heterotrophic denitrification.

Both in the laboratory and in the field, we observed a slight increase of sulphate concentrations, suggesting the occurrence of autotrophic denitrification by sulphide oxidation. Nevertheless, this type of denitrification seems to contribute only slightly to nitrate removal in comparison with heterotrophic processes.

Although we obtained the total removal of nitrate in the laboratory, denitrification was never complete in the field. Two major factors can explain the limited decrease of nitrate concentrations observed in the field, (i) the occurrence of nitrification processes and (ii) the limited availability of N and C substrates, since the field denitrification processes occur in localised anaerobic microsites of the unsaturated zone, while, in the laboratory experiments, all the samples were saturated with water allowing optimal diffusion.

This investigation of biogeochemical activities in the vadose zone provided some general information on the magnitude of the denitrification occurring in unsaturated weathered granite and on the hierarchy between the different denitrifying processes. It would be interesting to characterise the diversity of the microorganisms responsible for nitrate removal in order to explain the spatial and temporal variability of the denitrifying activity.

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