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Reactive modeling of denitrification in soils with natural and depleted organic matter

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1 **ABSTRACT**

2

3 Nitrogen fertilizers used in agriculture often cause nitrate leaching towards shallow
4 groundwater, especially in lowland areas where the flat topography minimize the surface
5 run off. To introduce good agricultural practices in order to reduce the amount of nitrate
6 entering the groundwater system, it is crucial to quantify the kinetic control on nitrate
7 attenuation capacity. With this aim a series of anaerobic batch experiments, consisting of
8 loamy soils and nitrate contaminated groundwater, were carried out using acetate and
9 natural dissolved organic matter as electron donors. Acetate was chosen because it is the
10 main intermediate species in many biodegradation pathways of organic compounds, and
11 hence is a suitable carbon source for denitrification. Sorption of acetate was also
12 determined, fitting a Langmuir isotherm in both natural and artificially depleted organic
13 matter soils. To account for the spatial variability of soil parameters, experiments were
14 performed in quadruplicate. The geochemical code PHREEQC(2) was used to simulate
15 kinetic denitrification using Monod equation, equilibrium Langmuir sorption of acetate and
16 equilibrium reactions of gas and mineral phases (calcite). The reactive modeling results
17 highlighted a rapid acetate and nitrate mineralization rate, suggesting that the main
18 pathway of nitrate attenuation is through denitrification, while calcite acted as a buffer for
19 pH. While, in absence of acetate the natural content of organic matter did not allow to
20 complete the denitrification process leading to nitrite accumulation. Reactive modeling is
21 thought to be an efficient and robust tool to quantify the complex biogeochemical reactions
22 which can take place in underground environments.

23

24 **KEYWORDS**

25 Denitrification, soil, acetate, reactive modeling.

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1. Introduction

Nitrate (NO_3^-) is a widespread inorganic pollutant in shallow groundwater aquifers due to agricultural fertilization ([Galloway et al. 2008](#); [Rivett et al. 2008](#)) and other sources, like industrial and municipal sewer systems ([Wakida and Lerner 2005](#)). NO_3^- concentrations are often found spatially and temporally variable in aquifers ([Böhlke et al. 2002](#); [Wriedt and Rode 2006](#); [Thayalakumaran et al. 2008](#)), this is usually related to variations in groundwater flow and nitrate attenuation rate ([Tesoriero et al., 2000](#); [Almasri and Kaluarachchi, 2007](#)). In Italy, the Po River valley is the largest and more intensively farmed alluvial plain, and is heavily impacted by NO_3^- groundwater contamination ([Mastrocicco et al., 2010](#); [Onorati et al., 2006](#); [Cinnirella et al., 2005](#)) and surface water eutrophication ([Provini et al., 1992](#); [Palmieri et al., 2005](#)).

Nitrogen attenuation from surface to groundwater systems may occur via bacterial heterotrophic denitrification, using NO_3^- as electron acceptor and a carbon (C) source as electron donor producing nitrogen gases ([Coyne, 2008](#); [Schipper et al., 2008](#)). This process has been extensively studied in superficial ecosystems ([Seitzinger et al. 2006](#)) but not often with reference to the complexity of agricultural practices ([Barnes and Raymond, 2010](#)). Soil type and tillage, crops, irrigation techniques and types of nitrogen fertilizers form a variety of terms emphasizing site specificity of denitrification and consequently the risk of nitrogen leaching towards groundwater ([Kay et al., 2009](#)). Acetate is the main intermediate species in many biodegradation pathways of organic compounds, and hence is a suitable carbon source for denitrification ([Strobel,2001](#); [Baker and Vervier, 2004](#)). However, the acetate denitrification efficiency is likely to be related to sorption ([Jones and Brassington, 1998](#)).

1 In the recent past, to understand the various chemical and biological processes
2 responsible for the natural reduction of nitrate in groundwater, a number of laboratory
3 batch experiments and reactive-transport modeling has grown. Numerical models are
4 routinely configured to incorporate large numbers of integrated physical, geochemical and
5 microbiological processes controlled by kinetic and equilibrium conditions ([Barry et al.
6 2002; Brun and Engesgaard 2002; Prommer et al. 2009](#)).

7 The purpose of this research was to investigate the importance of soil organic matter
8 (SOM) content on denitrification, in NO_3^- contaminated groundwater, by using laboratory
9 batch reactors simulated via the reactive geochemical code PHREEQC(2) ([Parkhurst and
10 Appelo, 1999](#)). Specifically, this study investigated the occurrence of denitrification both
11 with excess of acetate and in limitation of organic substrate using natural soils and
12 artificially SOM's depleted soils.

13

14 **2. Materials and Methods**

15

16 **2.1 Soil and groundwater characterization**

17

18 Silty-loam sediments, here referred as Reference Soil (RS), were collected in four different
19 location within a maize plot using an Eijkelkamp hand driven soil sampler (Giesbeek, The
20 Netherlands) at a depth from 1.5 to 2.0 m below ground level (b.g.l.), stored in closed
21 refrigerated containers and immediately transferred in the laboratory. The sediments
22 belong to an agricultural field located in the alluvial plain of the Po river (Ferrara, Italy);
23 details on crop rotation and site conditions are given in [Mastrocicco et al., 2010](#).

24 Groundwater was collected by PVC piezometers installed in the same drilled holes where
25 the sediments were collected; the piezometers were screened in the last 10 cm of the PVC
26 tube and groundwater was collected via low flow method with an inertial pump to preserve

1 natural conditions. The static water table was at 1.3 m b.g.l., thus the sediments were fully
2 saturated and in equilibrium with groundwater.

3 Grain size distribution (GSD) of the soil samples was obtained using a tested and
4 calibrated settling tube ([Brambati et al., 1973](#)) for the sandy fraction and an X-ray
5 Micromeritics Sedigraph 5100 for the finer fraction ([Artigas et al., 2005](#)). Bulk density (ρ)
6 and volumetric water content (θ) of the soil, were measured gravimetrically. Soil pH was
7 determined using 1:1 soil/water ratio.

8
9 Table 1: Average GSD, ρ , θ and SOM measurements for RS, the symbol \pm expresses the
10 standard deviation on four replicates.

Parameter	RS
Grain size (%)	
Coarse sand (630-2000 μm)	0.3 \pm 0.0
Medium sand (200-630 μm)	3.5 \pm 0.2
Fine sand (63-200 μm)	7.0 \pm 0.3
Silt (2-63 μm)	59.6 \pm 0.4
Clay (< 2 μm)	29.6 \pm 0.6
ρ (Kg/m ³)	1.48 \pm 0.1
θ (-)	0.42 \pm 0.1
SOM (%)	2.2 \pm 0.2

11
12 SOM content was measured by L.O.I. method ([Tiessen and Moir, 1993](#)). To remove the
13 SOM, 200 g of the soil samples were treated at room temperature using 10 repeated
14 washes in hydrogen peroxide at 30% in volume. The procedure used to remove SOM was
15 developed at 20 \pm 0.5 °C in order to minimize the mineral and amorphous phases alteration

1 ([Mikutta et al., 2005](#)). The samples obtained are artificially depleted in organic matter and
2 here are referred as Depleted Soil (DS).

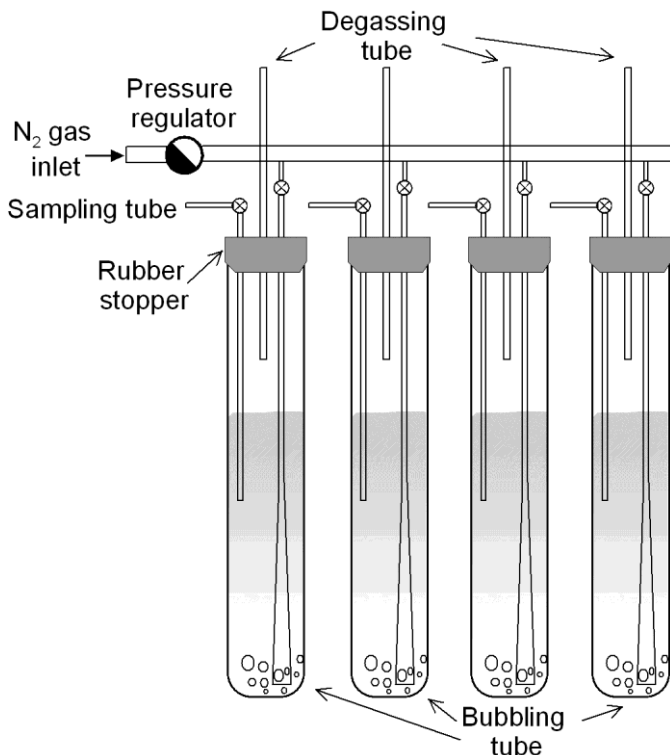
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4 **2.2 Batch experiments set up**

5

6 Microcosms were constructed out of 20 g of soil and 100 ml of groundwater in 250-ml U-
7 bottom shaped bottles capped with rubber stopper valves. All microcosms were incubated
8 in the dark using aluminum foils to cover them. To assure anaerobic conditions, the
9 reactors were continuously purged with N₂ gas via a multi inlet parallel system keeping a
10 constant pressure of 1.1±0.01 atm with a high precision manometer (Figure 1). To allow
11 for large bubbles to be formed at the base of the reactor, a conical PE tube was employed.

12



13

14 Fig. 1: The experimental set up, with a set of four replicates for each treatment; cross-
15 circles symbols represent HDPE switch valves.

16

1 The room temperature was adjusted to reach the target temperature of 15.0 ± 0.2 °C within
2 the microcosms. The N₂ gas bubbles provided an homogeneous mixing inside the reactors
3 and keep the temperature constant throughout the experiment, because the gas
4 expansion leads to a decrease of temperature necessary to balance the temperature
5 increment due to the heat loss from the magnetic plate of the stirrers. The agitation rate
6 was maintained not extremely high to avoid turbulence but high enough to produce a
7 homogeneous suspension in order to exclude the grinding effect that can eventually
8 disturb the microbial community. Table 2 summarizes the microcosm conditions selected
9 to evaluate the effect of SOM on denitrification rate. The microcosms were prepared in
10 quadruplicate, and acetate was added in stoichiometric excess to allow complete nitrate
11 removal.

12

13 Table 2: Microcosm for the evaluation of the influence of SOM content on the
14 denitrification kinetic.

Type	Microcosm components
GW	Groundwater
RS-GW	Groundwater, Reference Soil
DS-GW	Groundwater, Depleted Soil
RS-Ace	Groundwater, Reference Soil, acetate 5.5 mM
DS-Ace	Groundwater, Depleted Soil, acetate 5.5 mM

15

16 **2.3 Analytical methods**

17

18 Technical grade (99% purity) acetate was purchased from Merck (Darmstadt, Germany).
19 Samples were filtered through 0.22 µm Dionex vial caps. The major cations and anions
20 together with acetate were determined by an isocratic dual pump ion chromatography ICS-

1 1000 Dionex, equipped with an AS9-HC 4 x 250 mm high capacity column and an ASRS-
2 ULTRA 4mm self-suppressor for anions and a CS12A 4 x 250 mm high capacity column
3 and a CSRS-ULTRA 4mm self-suppressor for cations. The eluent used for anions
4 analyses was sodium carbonate 18 mM, while eluent used for cation analyses was
5 metanesulphonic acid 9 mM; flow rate was set to 1.0 ml/min for both anions and cations
6 analyses. An AS-40 Dionex auto-sampler was employed to run the analyses, Quality
7 Control (QC) samples were run every 10 samples. The standard deviation for all QC
8 samples run was better than 4% relative. Dissolved ferrous iron (Fe^{2+}) was measured
9 using a Hach DR/890 portable colorimeter. Charge balance errors in all analyses were
10 less than 5% and predominantly less than 3%. Alkalinity content was determined using a
11 Merk Aquaquant titration package. Water used was Milli-Q grade (Millipore, MA, USA).
12 Dissolved organic carbon (DOC) was determined with a carbon analyzer (Carbon Analyzer
13 Shimadzu TOC-V-CSM) after acidification with one drop of 2 M HCl to remove dissolved
14 carbonate. Calcite and dolomite content in soil were determined with a Chittick
15 gasometrical apparatus ([Dreimanis, 1962](#)).

16 Bacterial growth was measured by monitoring culture turbidity as absorbance at 550 nm
17 with a Jasco 550 UV/Vis, double beam spectrophotometer. The absorbance readings,
18 corrected by difference with a reference sample of the same sterilized ground water, were
19 used to calculate biomass concentrations from a calibration curve obtained with measured
20 concentrations of heterophilic bacteria ([Peyton et al., 2001](#)).

21

22 **2.4 Acetate adsorption isotherm determination**

23

24 Sorption isotherm was measured for acetate in triplicate for both the RS and the DS. In a
25 plastic vial of 10 ml, 5 ml of acetate solution was mixed with 1 g of dry soil sterilized via 0.2
26 mM of sodium azide (solid:water ratio 1:5).

1 The initial acetate solution concentrations were 0.02, 0.2, 0.5, 1.0, 1.5 and 2.0 mM,
2 following the soil addition the samples were shaken for 10 min on a reciprocating shaker
3 operating at a speed of 250 rpm. The samples were subsequently centrifuged (10.000
4 rpm, per 15 min) and the supernatant solution recovered and analyzed. The acetate
5 amount recovered after equilibration was subtracted to the amount of acetate in the
6 original solution, giving the acetate adsorbed into soil. The experimental sorption of
7 acetate data was interpreted using the classical Langmuir isotherm ([Appelo and Postma,](#)
8 [2005](#)):

$$9 \quad C_s = \frac{S_{max} K_L C_l}{1 + K_L C_l} \quad (1)$$

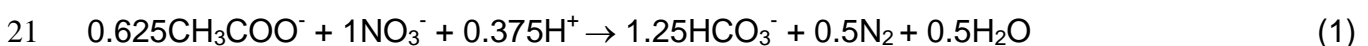
10 where C_s (mg/kg) is the adsorbed concentration, C_l (mmol/l) is the liquid concentration, K_L
11 (l/mmol) is the Langmuir coefficient (binding constant), and S_{max} (mmol/kg) the saturation
12 capacity. K_L and S_{max} , were determined by non-linear least squares data fitting.

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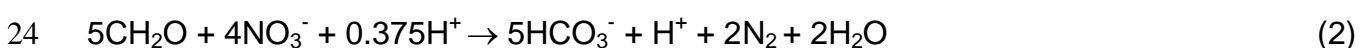
14 **2.5 Reactive modeling**

15

16 The USGS geochemical speciation model PHREEQC(2) ([Parkhurst and Appelo, 1999](#))
17 was used to simulate the titration and subsequent complete mineralization of acetate
18 under nitrate reducing conditions into the microcosms, as described in the simplified
19 stoichiometry equation for the energy-producing part of the denitrification reaction using
20 acetate as the electron donor:



22 While in presence of soil organic matter, when acetate was not supplied, the simplified
23 stoichiometry equation reduced to:



1 Since during the experiments nor manganese nor iron and sulphate reduction took place,
 2 denitrification was the only relevant terminal electron acceptor process. The kinetic
 3 degradation of organic matter (generic and acetate) was modelled on the basis of the
 4 Monod equation with microbial growth and decay ([Clement et al., 1997](#)):

$$5 \quad \frac{\partial C_S}{\partial t} = V_{max} \frac{C_S}{(C_S + K_{hS} C_S)} \frac{C_{NO_3^-}}{(C_{NO_3^-} + K_{hN} C_{NO_3^-})} C_M \quad (3)$$

$$6 \quad \frac{\partial C_M}{\partial t} = V_{max} Y - b C_M \quad (4)$$

7 where V_{max} is the acetate maximum uptake rate (Mol/l/s), C_S (Mol/l) is the acetate
 8 concentration utilized by bacteria as electron donor, K_{hS} is the substrate half saturation
 9 constant (Mol/l), $C_{NO_3^-}$ (Mol/l) is the nitrate concentration utilized by bacteria as electron
 10 acceptor, K_{hN} is the nitrate half saturation constant (Mol/l), C_M is the microbial
 11 concentration (Mol cells/l), Y is the microbial yield, namely the ratio of microbes grown to
 12 substrate utilized (Mol cells/Mol substrate); and b is the first-order decay rate of the
 13 microbial population (1/days). The equations 2 and 3 are nonlinear and coupled, therefore
 14 they must be solved iteratively. The parameters for the microbial processes were fitted
 15 with the experimental data. The parameters that had to be fitted are V_{max} , K_{hS} and Y for
 16 both acetate and DOC and K_{hN} for NO_3^- . Since the experiments were conducted over a
 17 relatively short time and the microbial population was in the exponential growth phase, it
 18 was assumed that b coefficient could be set to zero ([Schirmer et al., 1999](#)). Following this
 19 assumption Y was calculated dividing the concentration of biomass per the mass of carbon
 20 source used during the denitrification process.

21 The initial water, gas and immobile phases composition, is given in Tables 3 and 4. The
 22 equilibrium constants were taken from the PHREEQ-C database.

23
 24 Table 3. Average groundwater chemistry, concentrations in mmol/l.

Parameters		Parameters	
Temp. (°C)	15.0±0.2	NO ₂ ⁻	0.1±0.1
pH	7.6±0.1	NO ₃ ⁻	4.7±0.1
Eh (mvolt)	40.0±5.0	Ca ²⁺	6.2±0.2
Alkalinity	3.0±0.2	Mg ²⁺	2.8±0.2
O ₂	3.6±0.3	Na ⁺	3.1±0.3
Acetate	0.0±0.0	K ⁺	0.1±0.1
Cl ⁻	1.0±0.3	SO ₄ ²⁻	6.7±0.4
NH ₄ ⁺	0.0±0.0	PO ₄ ³⁻	0.0±0.0

1

2 The model also accounted for the secondary reactions that were triggered, for example by
3 the production of carbon dioxide and the protons utilization or release, both resulting from
4 the mineralization of acetate. These secondary reactions cause a shift of the calcite
5 equilibrium.



7

8 Table 4: Initial concentration of immobile phases (Mol/l) and gases partial pressure (Atm)
9 for each microcosm.

Constituent	Type	RS-Ace	DS-Ace	RS-GW	DS-GW	GW
CO ₂	Gas	0	0	0	0	0
N ₂	Gas	1.1	1.1	1.1	1.1	1.1
H ₂	Gas	0	0	0	0	0
H ₂ O	Gas	0	0	0	0	0
Calcite	Immobile	5e-2	5e-2	5e-2	5e-2	0
Biomass	Immobile	1e-4	1e-6	1e-4	1e-6	1e-6

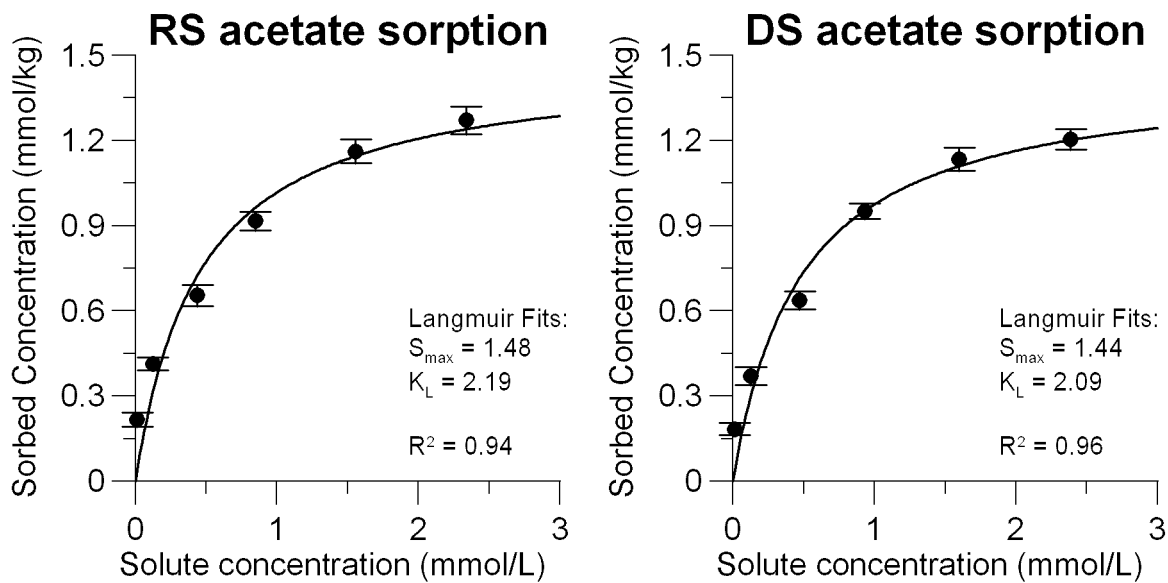
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Additionally, the gas exchange processes were modeled, for N₂, H₂ and CO₂, at fixed pressure of 1.1 atmospheres and variable volume, to allow degassing when the partial pressure of a specific gas exceed the fixed pressure. Finally, the adsorption-desorption of acetate on soil particles was modeled with the Langmuir equation (see Par. 2.3), using the parameters K_L and S_{max} , independently, obtained by non-linear least squares data fitting for both RS and DS. The Langmuir equation was implemented in PHREEQC(2) via the surface-complexation data block.

3. Results and Discussion

3.1 Acetate adsorption experiments

Figure 2 shows the fitted Langmuir equation to the observed acetate concentration. Generally, the data matched well to the Langmuir equation with R² values exceeding 0.94 for both RS and DS. The degree of sorption to the soil's solid phase was limited in both the examined sediments and found quite similar, although the DS exhibited an even lower maximum sorption capacity and Langmuir coefficient with respect to the RS. The lower sorption capacity was probably due to the loss of organic carbon from sediment that usually contribute to the bulk sorption capacity ([Karickhoff, 1984](#)). The treatment with H₂O₂ destroyed approximately the 42±5% of SOM, but the magnitude of sorption decrease was very low in comparison with the loss of SOM. Thus, it was concluded that for these sediments acetate sorption is not greatly affected by the SOM content, conversely absorption onto mineral surfaces might play the major role.



1

2 Fig. 2: Plots of concentration dependent sorption of acetate in the reference soil (RS) and
 3 in the organic matter depleted soil (DS). Symbols denote experimental points while the
 4 curves represent Langmuir isotherms fitted to the experimental data. Error bars show the
 5 standard deviation of four replicates.

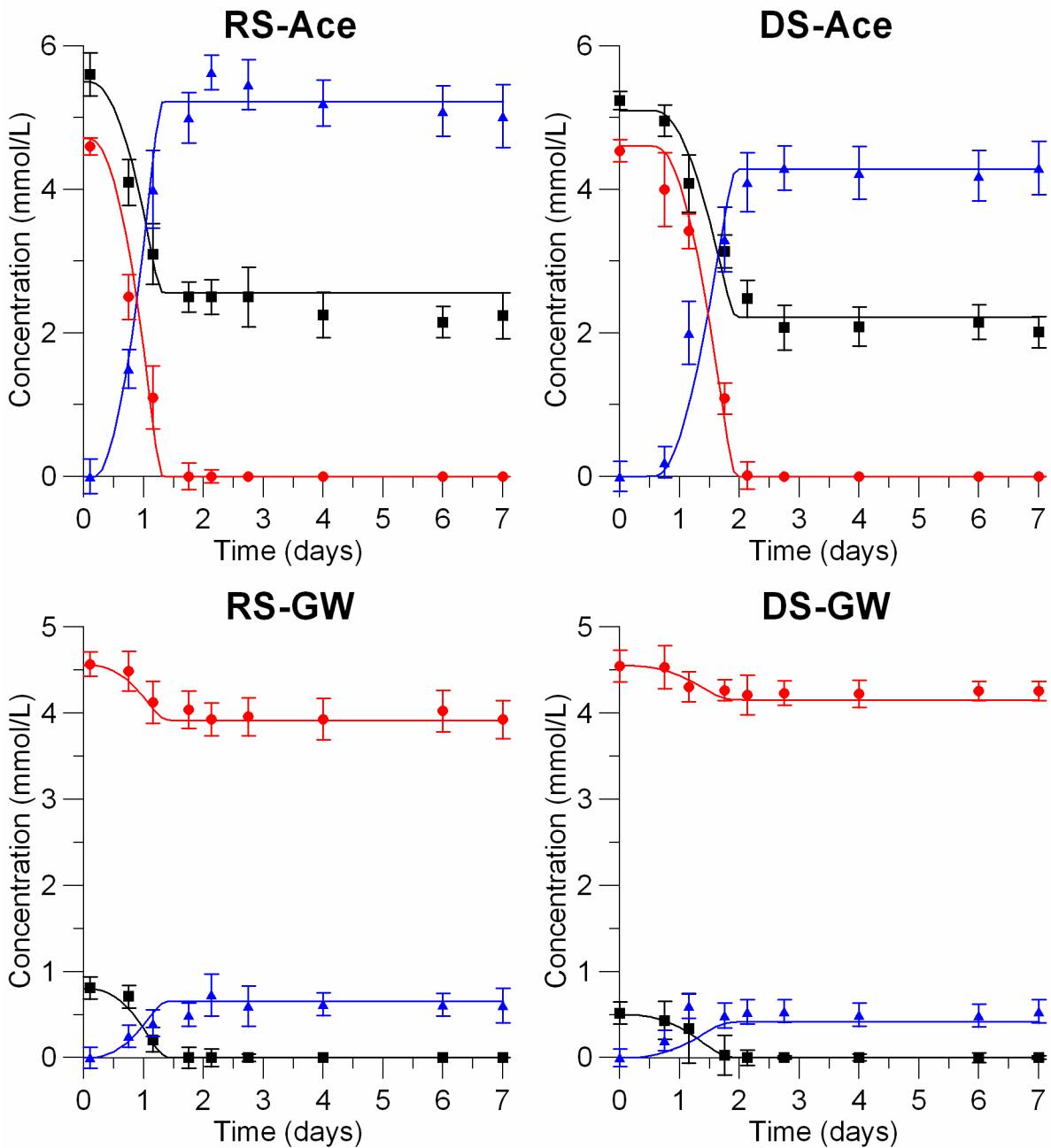
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7 3.2 Reactive modeling

8

9 Figure 3 illustrates that the model results fit well with the trends in the observed species
 10 concentrations and their changes during the experiments. The primary electron donor
 11 (acetate for RS-Ace and DS-Ace microcosms and DOC for RS-GW and DS-GW
 12 microcosms) and the terminal electron acceptor (nitrate) calculated curves show a good
 13 overlap to the observed trends. Also the end product (alkalinity) is reasonably in
 14 agreement with the observed values, although in the RS-Ace microcosms it was slightly
 15 underestimated by the model between the day 2 and 3.

16

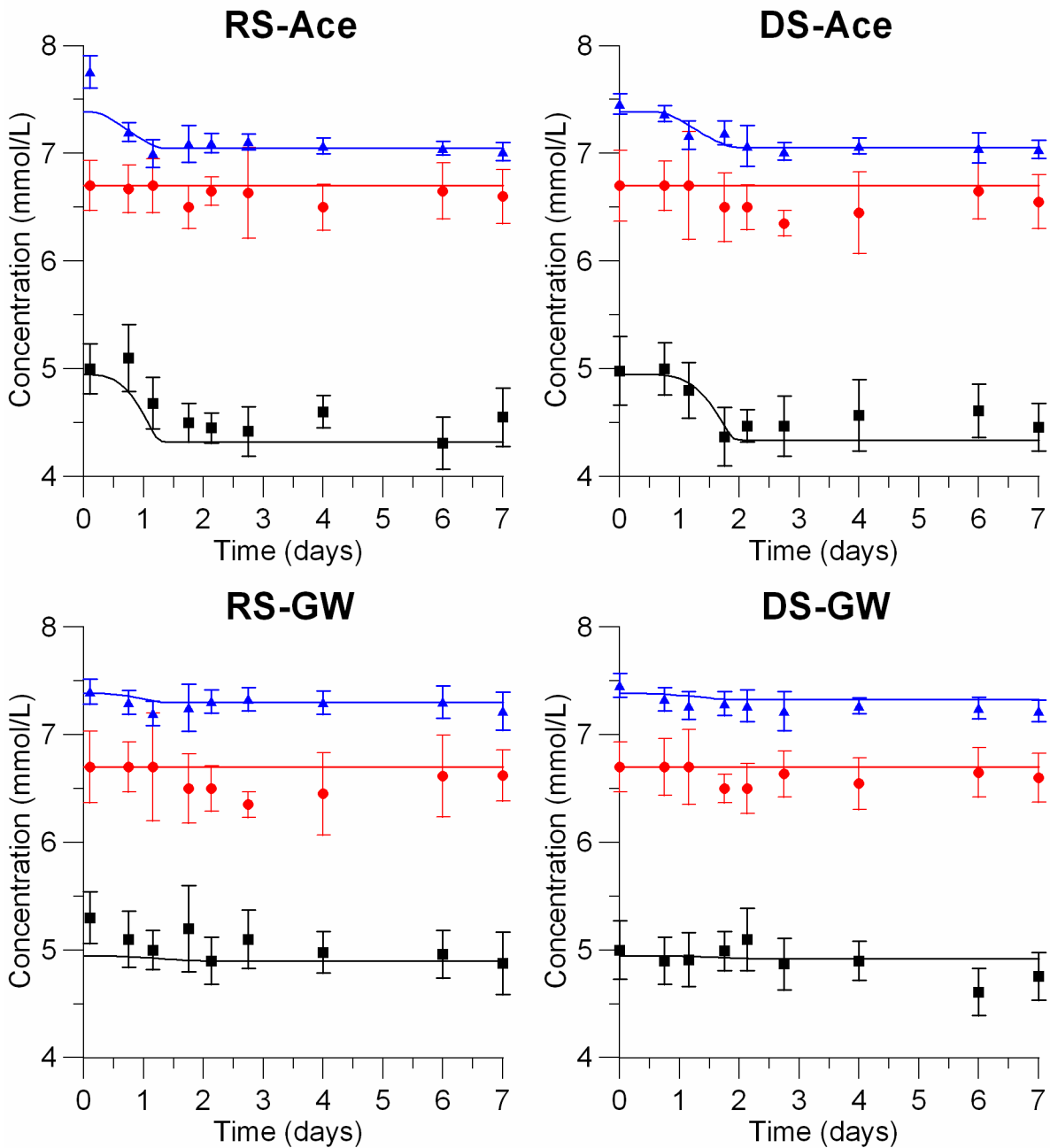


1

2 Fig. 3: Plots of PHREEQC(2) selected results for the microcosms. Symbols denote
 3 experimental points while the curves represent model results fitted to the experimental
 4 data. Black squares represent NO₃⁻, blue triangles alkalinity, red dots acetate (in the upper
 5 plots) and DOC (in the lower plots). Error bars show the standard deviation of four
 6 replicates.

7

1 The pH was nearly neutral in all the microcosms; in the RS-Ace and DS-Ace microcosms
2 the pH shift, from 7.6 to 7, was due to calcite precipitation; this was confirmed by calcium
3 (Ca^{2+}) decrease in solution during the denitrification phase (Fig. 4). The reactive model is
4 in agreement with observed Ca^{2+} concentrations, although in the RS-Ace microcosms the
5 calculated values slightly underestimated observed ones. Sulphate (SO_4^{2-}) remained
6 stable in all microcosms, despite a large variability shown by the error bars of figure 4. This
7 confirmed the lack of SO_4^{2-} reduction during the experiments and the absence of gypsum
8 precipitation as possible sink of dissolved Ca^{2+} .
9



1

2 Fig. 4: Plots of PHREEQC(2) selected results for the microcosms. Symbols denote
 3 experimental points while the curves represent model results fitted to the experimental
 4 data. Blue triangles represent the observed pH, red dots SO_4^{2-} , black squares Ca^{2+} . Error
 5 bars show the standard deviation of four replicates.

6

1 The highest removal rate of NO_3^- was observed for RS-Ace (Table 5), while no
 2 degradation was observed for the GW microcosms where NO_3^- concentration remained
 3 stable during the whole experiment (not shown).

4

5 Table 5: Model parameters for microbial degradation for each microcosm.

Parameter	RS-Ace	DS-Ace	RS-GW	DS-GW	GW
V_{max} (Mol/l/s)	$1.6e^{-6}$	$1.4e^{-6}$	$7e^{-7}$	$6e^{-7}$	0
K_{hSAce} (Mol/l)	$5e^{-4}$	$5e^{-4}$	-	-	$5e^{-4}$
K_{hSDOC} (Mol/l)	-	-	$1e^{-4}$	$1e^{-4}$	$1e^{-4}$
K_{hN} (Mol/l)	$1e^{-4}$	$1e^{-4}$	$1e^{-4}$	$1e^{-4}$	$1e^{-4}$
Y_{Ace} (-)	0.35	0.35	-	-	0
Y_{DOC} (-)	-	-	0.25	0.25	0

6

7 Table 5 shows that V_{max} decreases if the SOM is depleted, although the magnitude of this
 8 decrement is not elevated. In fact, V_{max} in the DS was approximately 17% less than in the
 9 RS when acetate was used as primary electron donor and approximately 15% less when
 10 DOC was used as primary electron donor. V_{max} was the most variable parameter during
 11 model calibration, while K_{hS} , K_{hN} did not affect significantly the model results.

12 Y was used as input parameter and not calibrated, the mean observed values for Y_{Ace} was
 13 $0.35(\pm 0.04)$ in both the RS and DS microcosms and the mean observed values for Y_{DOC}
 14 was $0.25(\pm 0.06)$ in both the RS and DS microcosms. The same Y values obtained for both
 15 RS and DS microcosms confirm that denitrifying bacteria are able to grow even on media
 16 depleted in organic matter. In preceding studies, denitrification rates in batch reactors with
 17 groundwater and soil matrix using acetate as carbon source were found to be in
 18 agreement with the ones reported in this study ([USEPA, 1993](#)).

19

1 3.3 Transient nitrite accumulation

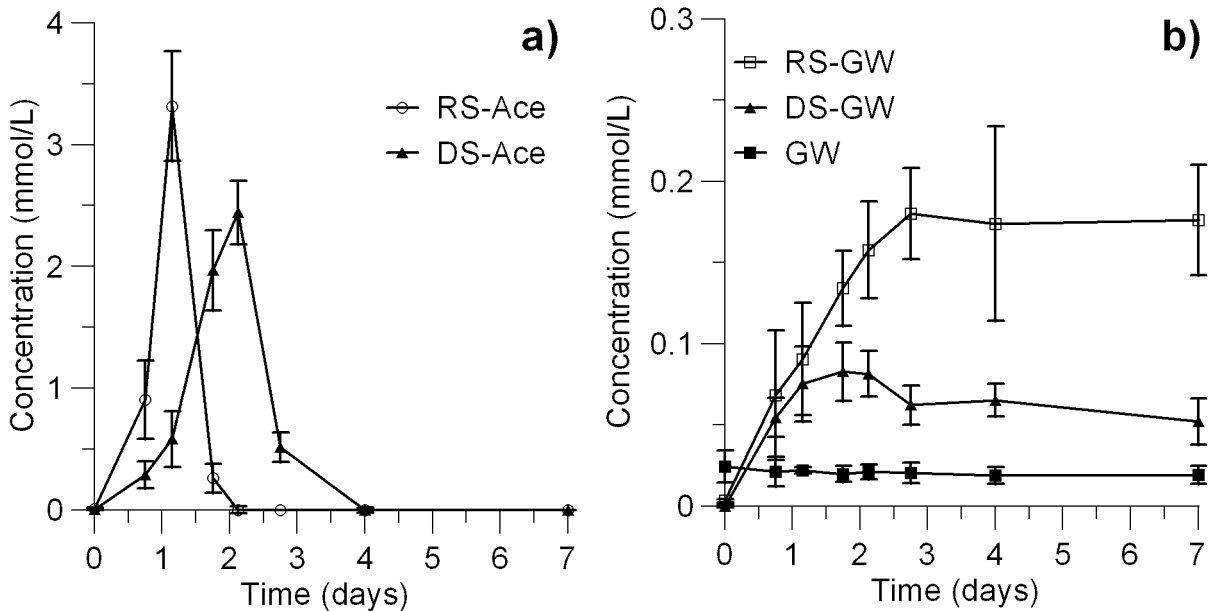
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3 During biological denitrification, nitrate is first converted to nitrite which is then further
4 reduced to N_2 gas through intermediates which generally are not rate limiting ([Soares,](#)
5 [2000](#)). Figure 5 shows that when acetate was used as primary electron donor, NO_2^-
6 accumulation took place both in the RS and in the DS microcosms, before the complete
7 depletion of NO_3^- as often observed in pure culture and in sludges ([Peyton et al., 2001;](#)
8 [Nair et al., 2007](#)). The mechanism of NO_2^- accumulation can be attributed to the transport
9 of the nitrite intermediate out of the bacterial cell which can occur when nitrate is available,
10 due to an inhibitory effect of nitrate on nitrite reduction. Using pure cultures, Kornaros et al.
11 (1996) and Rijn et al. (1996) observed that inhibition was dependent by the number of
12 electrons per mole of substrate compound, with greater accumulation of extracellular nitrite
13 with more oxidized substrates, like acetate, as used in this experiment. Suggested
14 mechanism implies that when substrate electrons are limited, nitrate is the preferred
15 electron acceptor compared with nitrite, via a competitive advantage for electrons of nitrate
16 reductase over nitrite reductase, as reported also by Thomsen et al., (1994) and Almeida
17 et al. (1995). As shown in Fig. 5, a, when NO_3^- substrate became depleted, NO_2^- reductase
18 repression ceased, leading to NO_2^- disappearance. The observed one day delay between
19 RS-ace and DS-ace is explained by the lag of bacterial growth, which in DS-ace, after
20 hydrogen peroxide treatment, had to start from the much lower concentration of ground
21 water.

22 A completely different trend was evidenced in GW, DS-GW and RS-GW microcosms,
23 where NO_2^- concentrations remained one order of magnitude lower and did not evidence
24 any late NO_2^- accumulation effect (Fig. 5). The increasing trend of concentration from GW,
25 to DS-GW and RS-GW can be attributed to the increasing availability of organic substrates

1 and the proportional stimulation of denitrification which is reflected in nitrite concentration;
2 these results are coherent with the ones shown in Fig.4.

3



4

5 Fig. 5: Plots of observed NO_2^- in the five microcosms described in Table 2. Symbols
6 denote experimental points while the curves are added to easily follow the trend. Error
7 bars show the standard deviation of four replicates.

8

9 4. Conclusions

10

11 The sorption capacity of acetate was weak in the investigated sediment as suggested by
12 others ([van Hees et al., 2003](#); [Fischer and Kuzyakov, 2010](#)) and was not substantially
13 reduced by the removal of organic matter. Sorption behavior was well reproduced by the
14 Langmuir equation for both the untreated (RS) and the treated (DS) sediments.

15 The reactive modeling described the complete nitrate mineralization in RS-Ace and DS-
16 Ace microcosms, in conjunction with the concomitant alkalinity increase. These evidences,
17 corroborated by the good model fit, indicated that the main pathway of nitrate attenuation
18 in these sediments may occur through denitrification in presence of acetate as electron

1 donor; while calcite acted as a buffer for pH. On the contrary, when a viable carbon source
2 is not available like in RS-GW and DS-GW microcosms, denitrification is likely to remain a
3 negligible pathway of NO_3^- removal from sediments. Overall, this study showed that in
4 SOM depleted sediments, NO_3^- natural attenuation could be comparable with non depleted
5 sediments if a viable carbon source is present. It follows that a rationale prevention,
6 management and mitigation of nitrate pollution, should focus on agricultural practices
7 which could increase the availability of labile organic matter and therefore augment the flux
8 of low molecular weight organic acids towards the sub soils. Modeling simple but
9 fundamental biological functions that regulate nitrate removal, as done in this study, may
10 contribute to the compilation of more representative nitrate transport reactive models in the
11 vadose zone.

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24 stata trovata. **References**

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