

# Subsurface nitrate reduction under wetlands takes place in narrow superficial zones

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**Keywords:** wetlands; nitrate reduction; organic matter; vertical distribution.

## Abstract

*The aim of the present study is to investigate the vertical distribution of the Nitrate Reduction Potential (NRP) at different depths on a natural (Evi site) and a re-established (Brynemade site) wetlands. The obtained NRP together with other environmental parameters provides a valuable data of the main driving factors affecting denitrification, the competitive process Dissimilatory Nitrate Reduction to Ammonium (DNRA) and the performance of a re-established wetland.*

*Intact soil cores (0-3 m) were collected and were divided in 10 cm slices for the determination of Organic Matter (OM) through Loss of Ignition (LOI) as well as Dissolved Organic Carbon (DOC) and Nitrate Reduction Potential (NRP) spiking nitrate in batch tests. The nitrate reduction was fitted as a pseudo-first order rate constant (k) from where NRPs were obtained.*

*Nitrate reduction took place in a very narrow superficial zone showing a dropping natural logarithmic trend along depth. The main driving factor of denitrification, besides depth, was OM. Although, DOC and LOI could not express by themselves and absolute correlation with NRP, high amounts of DOC ensured enough quantity and quality of labile organic matter for nitrate reduction. On the other hand, high concentration of recalcitrant LOI but a scarce abundance of DOC failed to drive nitrate reduction. DNRA showed to be important only in very superficial samples with high contents of OM. Lastly, the high nitrate reduction performance of the re-established wetland confirms that wetlands can be restored satisfactorily.*

## Introduction

Wetlands are examples of habitats that can be used directly to decrease nutrient loading of surface waters and to improve its quality. Nitrate Reduction (NR) in a riparian area is commonly attributed to denitrification, immobilization and plant uptake. [1, 2]

Specifically, denitrification is a biological process based on the biochemical reduction of nitrate and nitrite in groundwater thanks to the presence of anaerobe facultative bacteria in the site which can be autotrophic (if sulphur [3] or hydrogen [4] are present) or heterotrophic. In the latter case, electrons needed for nitrate reduction are originated from the oxidation of organic matter that also acts as a source of cellular carbon (heterotrophism). Illustrative, a stoichiometric reaction of a complete heterotrophic denitrification to nitrogen gas is presented in *Equation 1* [5].



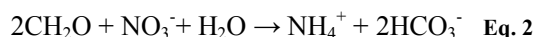
Riparian soils are rich in organic carbon substrate in top layers (Horizon O, e.g. Peat layer), that leaches dissolved Organic Matter (OM) which provides a carbon source to the heterotrophic denitrification pathway. In this way the age of the OM and the constant input of fresh OM from decayed vegetation is a key point for the process performance. The aging of (OM) has an important influence on the denitrification through soil depth because the disappearance of labile fraction against horizons and time, limits the labile leachate that reaches deeper horizons. [6, 7]

In addition, microorganisms are strongly influenced by environmental conditions: dissolved oxygen, mineralizable carbon, pH, nutrients (e.g. phosphorus) [8, 9], water table elevation [10, 11], temperature and other seasonal conditions, [12]. All these conditions are critical for the denitrification process. Moreover, environmental conditions in Northern European countries where low temperatures (around 10 °C) are common in groundwater might have a negative impact on denitrification performance since it is a temperature dependent process [13, 14].

Reported nitrate reduction rates ranged from 0.004 to 26.5 mg nitrate·l<sup>-1</sup>·day<sup>-1</sup> [15] and first order constants were in the range 3·10<sup>-5</sup> to 1.4 day<sup>-1</sup> [16] for different types of vegetal-based materials and under different conditions.

Due to high environment heterogeneity in riparian soils, past studies of spatial distribution of the denitrification potential in these systems showed high variations in width and depth, [17-21]. In addition to previously exposed factors in riparian zones, variability can be caused by the great diversity of minerals and organic materials that can be found in different stratigraphic positions, due to sediment transport and settling mechanisms. These mechanisms are affected by frequency and duration of floods, flow patterns, stream velocity and distance from the main channel and eventually sediments could be flushed at one place and filled at another. [22]

Dissimilatory Nitrate Reduction to Ammonium (DNRA) could also be present. DNRA is a respiratory or fermentative pathway where nitrate is reduced step by step to ammonium competing with denitrification for the nitrate in groundwater [23] and in riparian zones [24], *Equation 2*.



Ammonium is retained in the soil until nitrification, plant uptake or anaerobic ammonium oxidation process occurs (Anammox). DNRA are less understood than denitrification although it can be a significant or even a dominant process in some ecosystems, [25]. Conditions favouring DNRA may include high temperature [26] and high C/N ratio, [27]. It

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3 is believed, that heterotrophic denitrification supplies more free energy than DNRA, but  
4 under nitrate limiting conditions or high OM concentrations DNRA could be favoured  
5 because more electrons can be transferred per mole of nitrate, [28]. Notice that in both  
6 pathways nitrite is an intermediate.  
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9 In this work a lab-based methodology for the measurement of the Nitrate Reduction  
10 Potentials (NRP) on a natural and re-established wetlands at different depths is performed.  
11 The obtained NRP together with other environmental parameters provides a valuable data of  
12 the main factors affecting denitrification and the competitive process DNRA. It will validate  
13 the feasibility to restore wetlands with a working remediation role and assist the design of  
14 future re-established wetlands taking into account the key parameters affecting the  
15 denitrification performance.  
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## 17 18 **Materials and methods**

### 19 20 **Description of the sites**

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22 Two Danish wetland field sites with an extremely similar geology and climate were selected  
23 for the present study. The Brynemade site (Odense river basin in Odense Island) has been  
24 chosen as a model of a well re-established wetland and the Evi site (Holtum river basin in  
25 Jutland, Western Denmark) as a model natural wetland-river system.  
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29 The two sites present a very similar geology, both are glacial flood plains composed by a  
30 first layer of freshwater deposits such as peat, organic silt, clay and sand followed by a  
31 deeper layer of sand and gravel deposited by glacial streams from the Weichsel glacial  
32 period. Sites were previously characterized in terms of geology by means of drilling and  
33 borehole logging. Several monitoring campaigns were performed after the sampling of the  
34 cores in order to compose their respective hydrogeological models and to acquire  
35 information about the groundwater chemistry. [29-31]  
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38 Brynemade wetland at the Odense river Basin (Denmark) (55°13'12''N, 10°17'35''E;  
39 WGS84) was restored in 2003 and was used to document seasonal changes in flow  
40 dynamics and nitrate/pesticide degradation performance in a re-established wetland-river  
41 system. The restoration included re-raising the riverbed and re-meandering the river to the  
42 position it had prior to the 1958 channelization. The site is bordered by a Christmas tree  
43 plantation and crop land in the northern part.  
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46 The seasonal flow and transport of water and nitrates in this wetland was extensively  
47 modelled using hydrogeological characterization with wells, slug and infiltration tests,  
48 geophysical image of the subsurface using multi electrode profiling and measurements of the  
49 discharge to the river by seepage -meter and river bed temperature studies, [29]. From this  
50 modelling it was found that in non-flooding periods groundwater flow was horizontal from  
51 wetland to river. During flooding periods (75% of the year) flow was upward through the  
52 peat layer. It was estimated that between 25 and 37% of the total groundwater was  
53 discharged through the peat. The main climate parameters were an annual mean temperature  
54 of 8.9°C and an annual accumulated precipitation of 733 mm (Years: 2001 to 2010, grid  
55 20x20 km for temperature and 10x10 km for precipitation), [32].  
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3 Evi site is a natural wetland located close to Lake Ejstrup and the city of Ejstrupholm, in  
4 Jutland, (55°59'28''N, 9°19'43''E; WGS84). The catchment area is at the border of the  
5 maximum extension of the Weichselian glaciers corresponding to the last glacial advance.  
6 Unlike the Brynemade site, the Evi site is never flooded. Groundwater flow is strongly  
7 dependent on precipitations and no reverse flow from streambed to aquifer was observed,  
8 [30, 31]. The main climate parameters were an annual mean temperature of 8.4°C and an  
9 annual accumulated precipitation of 860 mm (Years: 2001 to 2010, grid 20x20 km for  
10 temperature and 10x10 km for precipitation), [32].  
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### 12 **Sampling of soil cores and characterization**

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15 Two intact soil cores from Brynemade (B C.1 and B C.2, up to 3 m) and Evi site (E C.1 up  
16 to 3 m and E C.2 up to 2 m) were collected in May 2012 and May 2013, respectively. The  
17 soil cores were divided in slices of 10 cm in the laboratory and these subsamples were  
18 handled under anaerobic (nitrogen glove box) conditions for determination of NRP.  
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21 Moisture (M) was determined gravimetrically as the loss of mass after drying at 105°C  
22 overnight according to the method ISO 11465:1993 values were expressed in wet basis.  
23 Loss of Ignition (LOI) was calculated as the fraction of dry matter that was removed after 16  
24 hours at 400 °C employing the standard method, [33].  
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27 Dissolved Organic Carbon (DOC) was determined at 1 hour batch experimental time and at  
28 the final of the test, the analyses were performed using a TOC analyzer (Multi N/C 3100)  
29 after being filtered through 0.2µm nylon filter, acidified with concentrated sulfuric acid and  
30 purged with synthetic air. These DOC values were used to estimate the initial concentration  
31 of DOC that could take part in nitrate reduction.  
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### 33 **NRP and DNRA of the soil cores**

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35 Batch tests were used to study the kinetics of NRP in subsamples. 10 g of soil from each  
36 subsample was mixed with deionised water (100 ml) and placed in 115 mL vials (97 mm in  
37 height, 48 mm in diameter). Vials were sealed with butyl rubber septums. The culture was  
38 enriched up to 25 mg·l<sup>-1</sup> of nitrate supplied by sodium nitrate stock solution. All procedure  
39 was performed in a globe box in a nitrogen atmosphere. Tests were incubated at 10°C in a  
40 non-stirred system until nitrate depletion or up to 42 days. During incubation, water samples  
41 were withdrawn periodically through the septum and inside the globe box to analyse  
42 inorganic nitrogen species (nitrate, nitrite and ammonium).  
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46 Concentration of nitrogen species in batches were determined by ion chromatography  
47 (Dionex ICS-2100). In particular, nitrate and nitrite were analysed by using the column Ion  
48 pack AS19 4x250mm (quantification limit for both analytes of 1 mg·l<sup>-1</sup>) and ammonium  
49 with the Ion pack CS16 4x250mm column (quantification limit of 0.2 mg·l<sup>-1</sup>). Concentration  
50 of DOC was determined by high temperature catalytic combustion – CO<sub>2</sub> NDIR-Detector.  
51 (Multi N/C 3100) (quantification limit of 0.2 mg·l<sup>-1</sup>).  
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54 First-order nitrate reduction rate constants (*k*) were determined by fitting the concentration  
55 results to *Equation 3*. Although the batches were analysed up to one month only the first  
56 days were considered in order to avoid side effects such as changes or contamination in  
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microbial populations. Quantification limit of  $k=0.01 \text{ d}^{-1}$  was considered by using this method.

$$\ln [C]_t = \ln [C]_0 - k \cdot t \quad \text{Eq. 3}$$

Where:

$[C]_t$  : Concentration of nitrate at time  $t$  ( $\text{mg}\cdot\text{l}^{-1}$ )  
 $[C]_0$  : Concentration of nitrate at time 0 ( $\text{mg}\cdot\text{l}^{-1}$ )  
 $k$ : nitrate reduction rate constant ( $\text{d}^{-1}$ )  
 $t$ : reaction time (d)

The generated data of  $k$  and characterization values have been treated by XLSTAT 2015.1.01 Addinsoft™ in order to perform Pearson Matrices.

Ammonium and nitrite were analysed in some depths in order to assess DNRA importance. Both, denitrification and DNRA have a first parallel step where nitrate is reduced to nitrite, but the important difference to determine DNRA is if finally nitrogen is present as ammonium or as gaseous nitrogen, *Equation 1 and 2*.

## Results and Discussion

### Characterization of cores

#### *Brynemade site*

At Brynemade site, based on the measurements of multi electrode profiling [29], the wetland hydrogeology could be characterized by a three-layer system, i.e., an upper 1-2 m thick peat layer followed by an approximately 8-18 m thick heterogeneous sand aquifer, and a lower more silty/clayey layer of an undetermined thickness. This conceptual model has been confirmed by the following core characterization.

The LOI content of the cores showed a top (0-100 cm) rich peat layer, with values close to 90% in B C.1 and 70% in B C.2., *Figure 1*. The deeper layers in B C.1 and B C.2 were inorganic, whereas in B C.2, the top peat was succeeded by a second layer (100-140 cm) of increased LOI content, averaging 13.8% (n=4).

Initial DOC, *Figure 1*, from lixiviates of B C.1 and B C.2 at 1 hour of running experiment presented high values in the upper part (0-100 cm) averaging 43.2 and 23.1  $\text{mg}\cdot\text{l}^{-1}$  (n=10, each core) respectively following a decreasing in a saw-tooth trend.

Final DOC from lixiviates (results not shown) were higher than initial ones for upper layers and were lower for deeper layers in B C.1, pointing to the peat as an slow releasing source of DOC obstructing stoichiometric study of denitrification. Probably, an excess of DOC was present in upper layers and denitrification was not limited by the substrate, completing all the reduction of the spiked nitrate. In the case of B C.2, the DOC concentration did not change during the experiment in any of the depths.



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3 Moisture showed similar behaviour with a very humid peat layer (70-80%) and deeper  
4 layers ranging between 10-20%, *Figure 1*. In this case there was a more progressive  
5 transition than was the case of LOI which could be due to capillary effects.  
6

#### 7 *Evi site*

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10 The LOI content of the cores from the Evi site (*Figure 2*) was very high. E C.1 had a  
11 relatively low concentration in the top layer (first 10 cm) with 17.1% followed by high  
12 organic content layer from 40 to 160 cm in depth averaging 76.9% (n=13) then a saw-tooth  
13 trend to extinction was observed. In E C.2 transitions between organic layers and mineral  
14 layers were very marked with a very rich top layer 0-30 cm averaging 73.7% (n=3) and  
15 other layer at 70-80 cm with 25.1% (n=1).  
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17  
18 Interestingly, initial DOC concentrations in both cores were very low in contrast to its LOI  
19 content, *Figure 2*. In E C.1 lixiviates were constant without a trend to extinction with depth,  
20 averaging  $14.7 \text{ mg}\cdot\text{l}^{-1}$  in all samples (n=29). Contrarily, E C.2 averaged very low  
21 concentrations. A sharp DOC decrease with depth was observed, from the upper 20 cm with  
22 a  $18.3 \text{ mg}\cdot\text{l}^{-1}$  to a base level of  $3 \text{ mg}\cdot\text{l}^{-1}$ . These values were appreciably lower than in the  
23 Brynemade site. No important DOC evolution was observed at the end of the experiment  
24 matching with the poor NRP observed.  
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27 Moisture values, such as those observed in Brynemade, were in accordance to LOI values,  
28 but in Evi the moisture was much lower than in Brynemade with an organic layer (more than  
29 10%) and deeper layers less than 10% moisture, *Figure 2*. As in Brynemade, the different  
30 materials suppose different moisture that could be linked to field capacity as deep levels  
31 were saturated with water.  
32

#### 33 **Nitrate reduction potential results**

34  
35 The nitrate concentration data in NRP experiments were fitted to *Equation 3* model.  
36 Examples of the fitting are shown in *Figure 3*. As it can be observed, nitrate reduction was  
37 explained as a pseudo first-order constant in the first days which is in agreement with the  
38 literature, [34]. Finally the lack of proper DOC for the nitrate reduction or the kinetic  
39 quantification limit of the technique restricted the application of the first-order model.  
40 Despite the low incubation temperature ( $10 \text{ }^{\circ}\text{C}$ ) used in order to simulate field conditions,  
41 high NRP were observed.  
42

43 At Brynemade site, high NRPs were found in the upper part of the cores <30 cm (*Figure 1*)  
44 that also showed high concentrations of LOI and DOC <70 cm. In this zone high first-order  
45 rate constants were achieved, reaching  $1.43 \text{ d}^{-1}$  B C.1 and  $1.49 \text{ d}^{-1}$  B C.2 in the top (0-10  
46 cm), then kinetics decreased exponentially with depth. Into these first 30 cm of soil NRP  
47 averaged  $1.05 \text{ d}^{-1}$  (n=3) B C.1 and  $0.70 \text{ d}^{-1}$  (n=3) B C.2. The high NRPs and rich OM zone  
48 was succeeded by a lower NRP and OM zone, nitrate reduction was undetectable,  
49 considered as  $0.01 \text{ d}^{-1}$ , in many samples under 70 cm depth and in most samples under 130  
50 cm depth giving an overall mean of  $0.14 \text{ d}^{-1}$  (n=31) 0 to 310 cm B C.1 and  $0.14 \text{ d}^{-1}$  (n=31) 0  
51 to 320 cm B C.2. A remarkably exception in B C.2 at 130-140 cm depth with  $0.63 \text{ d}^{-1}$  was  
52 found. Samples from below 315 cm would probably also be below the detection limit of  
53  $0.01 \text{ d}^{-1}$ . In addition, DOC was probably recalcitrant compared to the top layers because rate  
54 constants were negligible. The origin of this recalcitrant DOC in the cores was unlikely to  
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3 come from the sand and possible came from upper layers where the labile OM for nitrate  
4 reduction has been consumed.

5  
6 The Evi site showed a poor nitrate reduction potential in both cores in spite of high LOI  
7 content, even in the top zone of the soil (0-10 cm) being  $0.11 \text{ d}^{-1}$  E C.1 and  $0.18 \text{ d}^{-1}$  E C.2,  
8 respectively. The averages for the first 30 cm were:  $0.09 \text{ d}^{-1}$  (n=3) in E C.1 and  $0.11 \text{ d}^{-1}$  (n=3)  
9  $\text{d}^{-1}$  in E C.2. Then NRP followed a quickly trend to extinction also in E C.1 which presented a  
10 very high LOI content from 30 to 170 cm. Global means were  $0.04 \text{ d}^{-1}$  (n=29) 0 to 300 cm in  
11 E C.1 and  $0.03 \text{ d}^{-1}$  (n=20) 0 to 200 cm in E C.2, *Figure 2*.

### 12 13 14 15 **Link of NRP to environmental parameters**

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17 Cores from the same sites had a similar behaviour in NRP and had similar environmental  
18 parameters. Statistical analysis was performed merging the data from both cores of each site  
19 in order to unveil relevant correlations between the main parameters involved in the NRP.

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21 The Pearson Matrix (PM) *Table 1 (Brynemade) and 2 (Evi)* produces the correlation  
22 coefficients between all the couples of variables. This matrix has been extended by adding  
23 the natural logarithm of the measured variables to test linear, exponential and potential  
24 fitting models. For all these correlation studies, significance levels of 5% and 1% were  
25 calculated.

26  
27 From *Table 1*, all correlations in Brynemade exceeded 1% of significance level and showed  
28 a strong correlation between parameters. The most important correlations were the  
29 anticorrelations of soil depth (z) vs:  $\ln k$ ,  $\ln \text{LOI}$ ,  $\ln \text{DOC}$  and M demonstrating a natural  
30 logarithmic extinction through soil depth. It was also observed that  $k$ , LOI, DOC and M  
31 were positive and strongly correlated.

32  
33 The most important correlation found in Evi's PM, *Table 2*, were  $k$  vs  $\ln z$ . As in Brynemade  
34 all the measured parameters decrease with z. In parallel, only DOC-LOI,  $k$ -DOC, DOC-LOI  
35 and LOI-M correlations were significant.

36  
37 From statistical analysis, it was observed that the most relevant driving factors for  $k$  in  
38 Brynemade were  $\ln z$  and OM and in Evi  $\ln z$ .

39  
40 The link between NRP and OM (LOI and DOC) was only significant in the Brynemade site.  
41 In the Evi site NRP was lower than in Brynemade with high amounts of LOI but low DOC.  
42 This means that in this case the quality of the OM is more important than the quantity as  
43 stated in previous cases. [35-38]

44  
45 In order to compare the trends of  $k$  values with z, *Table 3* shows the fitting of  $k$  vs  $\ln z$  that  
46 was the best empirical correlation that fitted the trend of  $k$  measurements with z. As it can be  
47 seen, Brynemade showed much higher initial values of  $k$  (intercept is  $k$  value for 1 cm) with  
48 higher decreasing trend compared to Evi values.

### 49 50 51 52 53 54 55 **DNRA Evaluation**

DNRA evaluation was performed from the measurement of the increment of ammonium that is shown in *Table 4*.

As is seen in *Table 4*, B C.1 and B C.2 between 0-10 cm depth accumulated an important amount of ammonium. Nitrite in the same samples was negligible, with maximum increasing values of 1 mg nitrite·l<sup>-1</sup> in core B C.2 at 25 cm and without increase for cores B C.1, E C.1 and E C.2.

Taking into account that the nitrogen contained in an ammonium concentration of 7.2 mg·l<sup>-1</sup> is equal to 25 mg·l<sup>-1</sup> of nitrate (spiked initial concentration), the following expression could be used to evaluate the fraction of DNRA, *Equation 4*. *Table 5* shows the results of DNRA fraction.

$$f_{DNRA} = -3,472 \frac{\Delta[NH_4^+]}{\Delta C} \quad \text{Eq. 4}$$

Where:

$f_{DNRA}$ , DNRA fraction

$\Delta[NH_4^+]$  is the increment with time of ammonium concentration (mg·l<sup>-1</sup>)

$\Delta C$  is the variation in time of nitrate (mg·l<sup>-1</sup>) measured in the same points.

As is seen in *Table 5* the fraction of DNRA was only significant in the first 10 cm of Brynemade cores (between 43 and 50% in B C.2 and B C.1, respectively). Contrarily, at the Evi site, the DNRA activity was only important between 20 and 30 cm at E C.1, other samples always showed a DNRA fraction below 1%. These results could be explained by the high superficial concentration of organic matter that favours DNRA pathway.

## Conclusions

Nitrate reduction took place in a very narrow superficial zone showing a natural logarithmic decrease to extinction, hence being undetectable in the deepest samples. In addition, it is worth mentioning that nitrate reduction followed a pseudo first-order constant.

DOC and LOI could not express by themselves an absolute correlation with NRP, but high amounts of DOC during the experiments ensured enough quantity of labile organic matter for nitrate reduction. On the other hand, low concentrations of DOC even in a rich LOI soil presented low NRPs, strengthening the idea that labile OM (quality) is more important than quantity.

DNRA was only important in the very superficial samples of Brynemade, where a high content of labile OM could produce favourable conditions for DNRA.

Wetlands can be restored satisfactorily concerning nitrate reduction performance. Comparing a natural wetland (Evi) to the re-established (Brynemade) with a similar geology and climate and taking into consideration the high NRPs observed in the latter, it is clear that other factors besides the re-establishing condition play a more important role.

## Acknowledgments



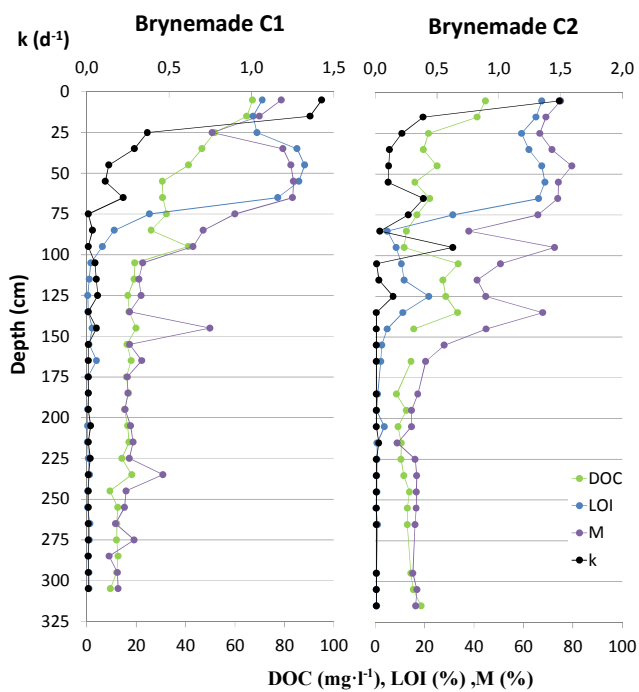
This work was funded by: the EU-project SQUAREHAB (FP7 - Grant Agreement Nr. 226565); the Spanish Ministry of Economy and Competitiveness through project ATTENUATION (CGL2011-29975-C04-03, Natural and Induced Attenuation of groundwater pollution from agricultural and industrial sources) and D Ribas is supported by Spanish Ministry of Economy and Competitiveness, under the FPI grant (ref. BES-2012-052327).

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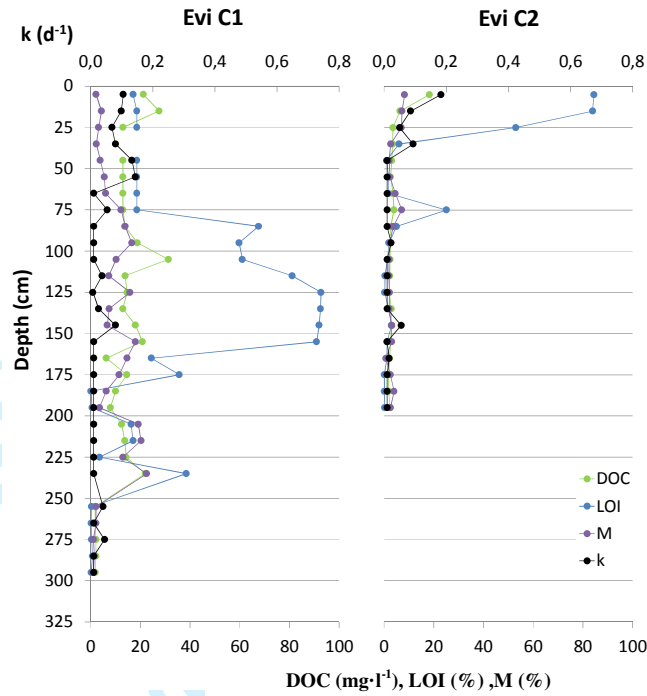
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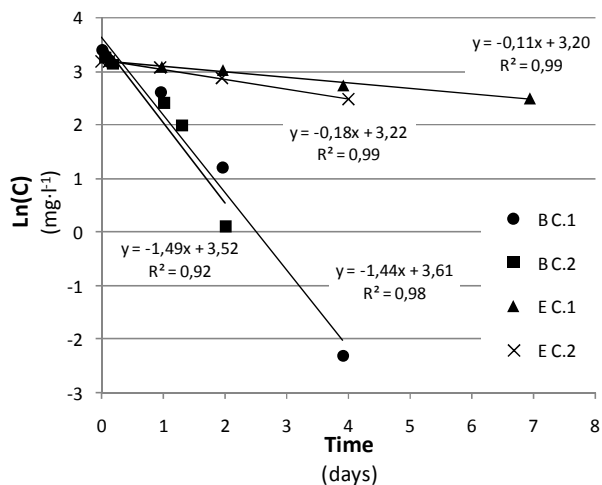


**Figure 1.** Depth-dependence of soil organic matter determined as DOC and LOI, soil moisture (M) and first-order nitrate reduction rate constants ( $k$ ) at the Brynemade cores.



**Figure 2.** Depth-dependence of soil organic matter determined as DOC, LOI, soil moisture (M) and first-order nitrate reduction rate constants ( $k$ ) at Evi Cores.





**Figure 3.** Kinetics fittings of first-order kinetics for 0-10 cm depth of Brynemade C.1 and C.2, Evi C1 and C.2.

**Table 1.** Matrix of Pearson correlation coefficients of Brynemade site Core 1 and 2 processed jointly, a statistical significance at the level of 5% is indicated in\* and at the level of 1% in\*\*. n = 60.

Variables	z	k	LOI	DOC	M	ln z	Ln k	ln LOI	ln DOC	Ln M
z	1,00									
k	-0,55**	1,00								
LOI	-0,78**	0,57**	1,00							
DOC	-0,70**	0,71**	0,71**	1,00						
M	-0,86**	0,55**	0,88**	0,68**	1,00					
ln z		-0,80**	-0,83**	-0,82**	-0,81**	1,00				
ln k	-0,80**		0,81**	0,67**	0,80**	-0,87**	1,00			
ln LOI	-0,87**	0,54**		0,71**	0,94**	-0,84**	0,79**	1,00		
ln DOC	-0,75**	0,60**	0,71**		0,73**	-0,79**	0,65**	0,74**	1,00	
ln M	-0,88**	0,51**	0,81**	0,69**		-0,80**	0,76**	0,92**	0,75**	1,00

**Table 2.** Matrix of Pearson correlation coefficients of Evi site Core 1 and 2 processed jointly, a statistical significance at the level of 5% is indicated in\* and at the level of 1% in\*\*. n = 49.

Variables	z	k	LOI	DOC	M	lnz	Ln k	lnLOI	lnDOC	Ln M
z	1,00									
k	-0,53**	1,00								
LOI	-0,28	0,24	1,00							
DOC	-0,26	0,31*	0,60**	1,00						
M	0,10	-0,15	0,53**	0,60**	1,00					
ln z		-0,72**	-0,26	-0,31*	0,14	1,00				
ln k	-0,50**		0,23	0,25	-0,21	-0,66**	1,00			
ln LOI	-0,49**	0,36*		0,70**	0,62**	-0,43**	0,35*	1,00		
ln DOC	-0,31*	0,33*	0,64**		0,67**	-0,33*	0,27	0,79**	1,00	
ln M	-0,10	-0,02	0,62**	0,65**		-0,02	-0,09	0,71**	0,75**	1,00

**Table 3.** Nitrate reduction potential first-order kinetic constants ( $k$ ) along depth fitted in natural logarithmic regressions. B (Brynemade), E (Evi), C (Core) and (n).  $z$  in cm.

Brynemade wetland		Evi wetland	
$k$ B.C1(d <sup>-1</sup> )	$k$ B.C2(d <sup>-1</sup> )	$k$ E.C1(d <sup>-1</sup> )	$k$ E.C2(d <sup>-1</sup> )
$-0.30 \cdot \ln(z) + 1.570$ R <sup>2</sup> = 0.71 (31)	$-0.23 \cdot \ln(z) + 1.241$ R <sup>2</sup> = 0.58 (29)	$-0.02 \cdot \ln(z) + 0.174$ R <sup>2</sup> = 0.48 (29)	$-0.03 \cdot \ln(z) + 0.198$ R <sup>2</sup> = 0.69 (20)

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**Table 4.** Measurements of ammonium ( $\text{mg}\cdot\text{l}^{-1}$ ) at the beginning (1h) and at the end of nitrate reduction experiments.

Depth	B C1		B C2		E C1		E C2	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0-10	1.8	6.0	3.0	6.2	0.6	0.9	0.5	0.8
20-30	0.6	1.0	0.6	1.0	0.3	0.7	< 0.2	< 0.2
40-50	0.4	0.2	0.2	0.2	0.3	0.4	< 0.2	< 0.2
180-190	<0.2	<0.2	< 0.2	<0.2	n.a.	n.a.	n.a.	n.a.

n.a. not available

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**Table 5.** Measurement of  $f_{DNRA}$  in reduction experiments.

Depth	B C.1	B C.2	E C.1	E C.2
0-10	0.50	0.43	0.04	0.04
20-30	0.05	0.06	0.12	<0.01
40-50	<0.01	<0.01	0.01	<0.01
180-190	n.a.*	<0.01	n.a.	n.a.

\*n.a.: not available

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