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Effect of different seasonal conditions on the potential of wetland soils for groundwater denitrification

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ABSTRACT

Wetlands, as active riparian areas in denitrification processes, are largely dependent on the environment. The main objective of this paper is to evaluate changes in the denitrification potential of wetland soils at laboratory scale promoted by climatic and seasonal influences. Several batch denitrification tests were performed with fresh wetland soil (peat) from Brynemade (Denmark) under: three different temperatures (20, 10, and 5˚C), drought period, and freeze-thaw event. Results show that nitrate was eliminated in all the experiments in percentages over 90%. However, not all the nitrate removed was reduced to nitrogen gas via the denitrification process; dissimilatory nitrate reduction to ammonium (DNRA) was also present. In fact, the percentage of total nitrogen eliminated at the end of the tests was: 79.7% at 20˚C, 84.1% at 10˚C, 82.9% at 5˚C, 41.0% in the dried soil, and 57.0% in the frozen soil. Thus, it can be concluded that the drying and freezing of the soil favor the DNRA process. Furthermore, in these conditions, nitrite increased sharply and was also accumulated possibly, as a DNRA or denitrification intermediate. Nitrate removal was fitted to a zero-order model, and an increase of the denitrification rates with the temperature was observed (3.8 mg NO_3^- L^{-1} d^{-1} at 20˚C, 3.0 mg NO_3^- L^{-1} d^{-1} at 10˚C, and 2.9 mg NO_3^- L^{-1} d^{-1} at 5˚C). These overall rates were modeled as a function of temperature by the Arrhenius equation and activation energy of 12.88 kJ mol^{-1} was determined. The fact that the activation energy is low in this work (unstirred batches) compared to previous publications (stirred batches) could be the result of a strong restriction on the nitrate mass transfer in the soil vs. reaction kinetics, which masks kinetic regulating factors of the denitrification rate. Thus, the variation of the denitrification rate with temperature is possibly the result of a combination of changes in mass transfer (diffusive transport) and kinetic constant variation, successfully modeled by the Arrhenius equation.

Keywords: Denitrification rates; DNRA; Temperature and seasonal effects

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1. Introduction

Basin waters are exposed to higher nitrogen inputs as a result of an increase in organic and chemical fertilizer usage in agriculture. This situation implies a risk of eutrophication and public health concerns resulting from nitrate and nitrite exposal. In order to counter this problem, natural wetlands, in the same way as riparian zones, play an important role in nitrogen remediation from nonpoint source pollution.

In spite of wetlands' theoretical performance, its denitrification potential shows an important variation with regards to many factors such as oxygen, organic carbon, temperature, nitrate concentration, and soil texture [1]. The relative importance of each one in limiting denitrification potential depends on its magnitude, finding in different cases, different principal limitation factors, that include: temperature [2], water table elevation [3], organic carbon [4], and nitrogen input [1].

Groundwater temperature is one of the most important factors controlling denitrification [5,6]. Like many other enzyme mediated biological reactions, denitrification shows a direct dependence with temperature, which could be modeled by employing the Arrhenius equation. Activation energy is positively related with the denitrification susceptibility to temperature. In addition to biologic aspects, dissolved oxygen is strongly negative depending on the temperature [7].

Periods of drought (seasonal or weather episodes) with the resulting loss of soil humidity have a deep impact on soil bacteria, nutrients, and texture. Due to the dry conditions, air enters into the soil and thus, soil becomes aerobic. Soil desiccation leads to larger bacterial mortality, which is reflected in the increase in dissolved carbon and exchangeable ammonium as a result of cell lysis and mineralization [8], the resulting ammonium could be transformed to nitrate enhancing in some cases the denitrification [9]. Once the soil is rehydrated, denitrifying communities gradually recover. A relation between the period of soil dehumidification and the period of the recovery of denitrification levels was found [10]. Additionally, there is a growing interest in this phenomenon because of the increase in N_2O emissions as a result of incomplete denitrification [11].

Freeze-thaw events release dissolved organic carbon (DOC) and nitrogen due to disruption of soil aggregates, plant residues, and lysis of microorganisms [12]. There is a so-called partial sterilization of denitrifying communities which causes an initial decrease in the denitrification rate but an increase in the growth of denitrifying bacteria that survived the freeze-thaw cycle [13]. Similarly to drought periods, freeze-thaw cycles increase the production of N_2O by denitrification, a process which is currently the object of numerous studies concerning climatic change [14].

Dissimilatory Nitrate Reduction to Ammonium (DNRA) is a respiratory or fermentative pathway where nitrate is reduced step by step to ammonium competing with denitrification for the nitrate [15], Eq. (1). Ammonium is immobilized in the soil until nitrification, plant uptake, or Anammox occurs. Conditions favoring DNRA are less understood than denitrification, although it can be a significant or even a dominant process in some ecosystems [16]. It is believed, however, that heterotrophic denitrification supplies more free energy than DNRA. Under nitrate limiting conditions, DNRA could be favored because more electrons can be transferred per mole of nitrate [17]. Notice that in both pathways nitrite is an intermediate.

$$NO_3^- \rightarrow NO_2^- \begin{cases} \rightarrow NH_4^+ & \text{DNRA} \\ \rightarrow NO \rightarrow N_2O \rightarrow N_2 & \text{Denitrification} \end{cases} \tag{1}$$

It is imperative to undertake further research into denitrification limiting factors because of its importance in the nitrogen cycle and nitrate bioremediation. In this paper, three extreme climatic conditions are studied: temperature variation, a period of drought, and a phase of freeze-thaw.

2. Methods

2.1. Site description

The site is a wetland of the Odense Rivern basin near Brynemade, Denmark (55°13′12″N, 10°17′35″E; WGS84) selected as a model of a well-established wetland. The "Brynemade" site has been carefully studied regarding geology, geochemistry, and geophysics in the framework of AQUAREHAB project [18]. The climate of the site is temperate and humid, characterized by windy winters and cool summers, an annual mean temperature of 8.9°C and an annual accumulated precipitation of 733 mm (Years: 2001–2010, grid 20 × 20 km for temperature and 10 × 10 km for precipitation) [19]. The site is used as pasture for livestock, such as horses.

2.2. Soil sampling and characterization

Peat soil (0–10 cm) was collected from a core extraction in May, 2012. The soil was for four months at field moisture and at 10°C in plastic bags. Soil samples were thoroughly homogenized under inert atmosphere and a small fraction was separated to perform an initial characterization: pH, electrical conductivity (EC), moisture, loss of ignition (LOI), and lixiviation test of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and DOC were performed and repeated in further characterizations. See results in Table 1.

Extractions with $CaCl_2$ (0.01 M) and de-ionized water (Milli-Q system) were prepared to measure pH and the conductivity of soil samples, respectively, according to the method UNE 77308:2001. Water content was determined as the loss of mass after drying at 105˚C overnight according to the method ISO 11465:1993. LOI was calculated as the fraction of dry matter that was removed after 16 h at 400˚C employing the standard method [20]. Nitrate, nitrite, and ammonium were extracted from soil samples with deionized water (dry soil:water ratio of 1:10 in weight) and further analyzed by ion chromatography (Dionex ICS-2100). The same lixiviate was also analyzed by TOC analyzer (Multi N/C 3100) to determine DOC content after being filtered through 0.2 μm nylon filter, acidified with concentrated sulfuric acid, and purged with synthetic air. All results are given in relation to dry weight.

2.3. Experimental setup

Three seasonal scenarios were identified as common environmental conditions in wetlands and were reproduced experimentally, as follows: temperature change, drought period, and a phase of freeze-thaw. Temperature change was tested culturing three identical batch lines spiked with 25 mg L^{-1} of nitrate at three different temperatures at 5, 10, and 20˚C, respectively. The drought period was created in the laboratory by maintaining the soils at 30˚C for one week, during which it lost most of the moisture. Meanwhile an aerobic condition as a result of atmospheric inlet was observed. Following that, the batches were setup, spiked with 25 mg L^{-1} of nitrate and maintained at 10˚C for the duration of the experiment. The freeze-thaw phase was studied by maintaining hydrated batch tests at −20˚C for one week and then thawed at room temperature (20˚C). Following that, batches were spiked with 25 mg L^{-1} of nitrate and maintained at 10˚C for the duration of the experiment. All conditions were tested three times, 10˚C was chosen as a reference temperature to compare between different conditions and periodically, batch tests were sampled for nitrate, nitrite, ammonium, and DOC analyses. Additionally, abiotic controls and negative controls without nitrate were performed.

2.4. Batch incubations

The equivalent of 2.92 g of dry soil and 100 mL of de-ionized water was placed in 115 mL vials (97 mm in height, 48 mm in diameter), in which settled soil a fine texture accounted for 10 mm. Vials were sealed with butyl rubber septums. The culture was enriched with 25 mg L^{-1} of nitrate supplied by sodium nitrate stock solution. All procedure was performed in a globe box in a nitrogen atmosphere. Vials were stored in thermostatic cabinets at target temperatures of 5, 10, and 20˚C under non-stirred conditions to simulate field conditions. All experiments were monitored until nitrate depletion fell to the minimum level of detection.

2.5. Analytical monitoring

Batches were analyzed periodically in approximately at 24 h intervals in order to follow the evolution of the aqueous substrates and intermediates of the denitrification pathways. Concentrations of nitrate, nitrite, and ammonium in the aqueous phase of the batches were determined by Ion chromatography (Dionex ICS-2100). The concentration of DOC was determined with a TOC analyzer (Multi N/C 3100) following the same procedure used for soil lixiviates.

2.6. Denitrification rate calculations

Denitrification rate could be calculated, thanks to nitrate consumption when nitrite remains below the

Table 1
Main physicochemical soil parameters and lixiviates concentration

Batch	pH	EC $_{25\ C}$ μS cm^{-1}	H (%)	LOI (%)	[NO_3^-] mg kg^{-1} d.m.	[NO_2^-] mg kg^{-1} d.m.	[$NH_4^+$$NH_4^+$] mg kg^{-1} d.m.	[DOC] mg kg^{-1} d.m.
Fresh soil	6.9	68.9	68.8	35.5	20.5	13.7	<Q.L	571.9
Dried soil	6.6	85.5	9.4	36.9	68.5	10.3	3.4	719.2
Frozen soil	6.6	74.2	–	–	27.4	30.8	<Q.L	547.9

Notes: d.m.: dry matter; <Q.L: below quantification level; – : not analysed.

level of detection. Results were tested in order to fit these models: first order, Monod [21], and zero order, finally the best fitting was obtained with zero-order model, Eq. (2).

$$[A]_t = [A]_0 - K \cdot t \tag{2}$$

where $[A]_t$ concentration of the nitrate at a particular time, $[A]_0$ initial nitrate concentration, K zero order rate constant, and t reaction time.

2.7. Arrhenius modeling

Nine zero-order rate constants at three different temperatures (20, 10, and 5˚C, respectively) were used to obtain activation energy and the frequency factor of the Arrhenius expression, Eq. (3), to model the zero order rate constant dependence on temperatures.

$$K_{(T)} = A \cdot e^{-\frac{E_A}{R \cdot T}} \tag{3}$$

where $K_{(T)}$ overall zero order rate constant at given temperature, ($\mathrm{mol\,L^{-1}\,s^{-1}}$(T)), A frequency factor, zero order ($\mathrm{mol\,L^{-1}\,s^{-1}}$), E_A activation energy ($\mathrm{J\,mol^{-1}}$), R universal gas constant (8.31 $\mathrm{J\,mol^{-1}\,K^{-1}}$), T absolute temperature (K).

Moreover, the Arrhenius equation was used to model the diffusion constants with temperature founding Arrhenius-type dependence [22], it is feasible because higher temperature leads to a higher average kinetic energy of molecules and higher collision rate.

3. Results

3.1. Soils characterization and lixiviates

Table 1 presents the results of the characterization of fresh soil, soil after a drought period, and soil after a freeze-thaw phase.

Fresh soil was characterized by an important organic fraction and a high water and slightly acidic content. Fresh soil lixiviated enough DOC to not be limited in heterotrophic denitrification pathway. As it is shown, dried peat can reach a higher amount of DOC, ammonium, and nitrate due to the cellular lysis as stated by some authors [10]. This table also shows that nitrate has increased as a result of preliminary aerobic mineralization. In the case of frozen soil, results shows few difference in DOC, contrarily to references [12] that found an increase of DOC owing to the cellular lysis and the disruption of soil aggregates.

3.2. Denitrification at different temperatures

Nitrate consumption could be fitted in a zero-order model at all temperatures (Fig. 1), obtaining the rate constants given in Table 2. As can be observed, nitrate consumption rates increased at a temperature of 20˚C when the process can be observed be quicker.

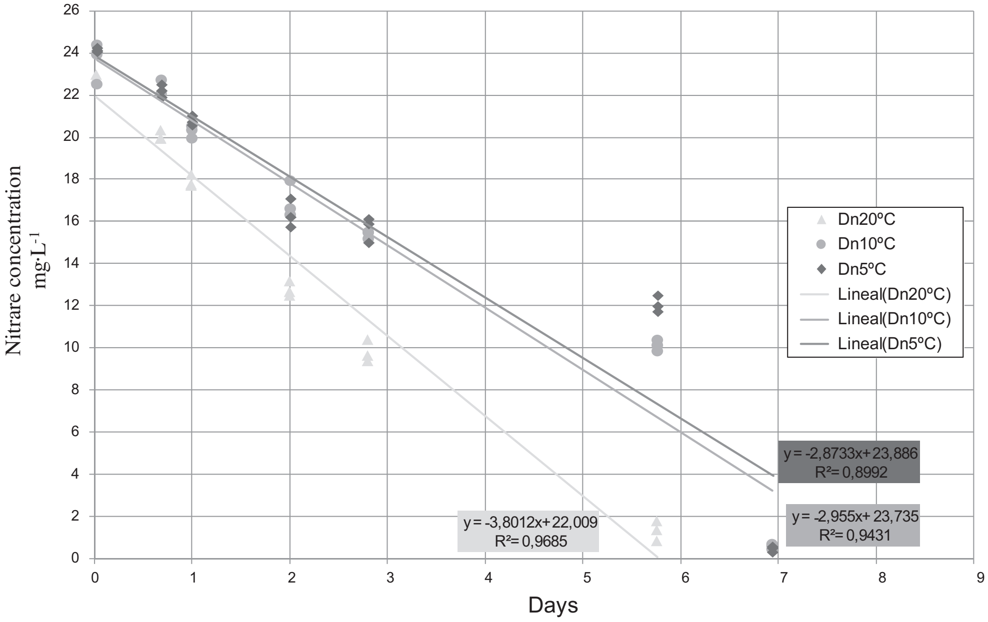

Fig. 1. Nitrate consumptions in batch at different temperatures and zero order model fits. Dn = Denitrification at ˚C.

Table 2
Observed rates at different temperatures

Temperature (°C)	Average rates ($mgNO_3^- L^{-1} d^{-1}$)	R^2 (n)
20	3.8 (± 0.10)*	0.97 (17)
10	3.0 (± 0.06)*	0.94 (20)
5	2.9 (± 0.01)*	0.90 (21)

*±95% confidence interval.

Table 3
Obtained Arrhenius equation constants

E_A ($kJ\,mol^{-1}$)	A ($mol\,L^{-1}\,s^{-1}$)
12.88 (± 1.07)*	6.12 (+3.48; −2.22)* 10^4

*±95% confidence interval.

Rate constants were used to obtain the activation energy and frequency factor from the Arrhenius equation (Table 3). With the determined constants of the Arrhenius equation, the overall rate of denitrification could be predicted in function of the temperature (Fig. 2).

The fact that the activation energy is low in this work (unstirred batches) compared to that which appears in published papers (stirred, column forced flow, or shaken) (Table 4), could be the result of a restriction on the nitrate transfer in the soil, which masks other regulating factors of the denitrification rate. Thus, the variation of the denitrification rate according to the temperature is suspected to be due to a combination of variation of mass transfer (diffusive transport) and kinetic constant variation, with a predominance of the transfer effect. Finally, despite the mix of both effects, its shared Arrhenius behavior could explain the observation of the expected match (Fig. 2).

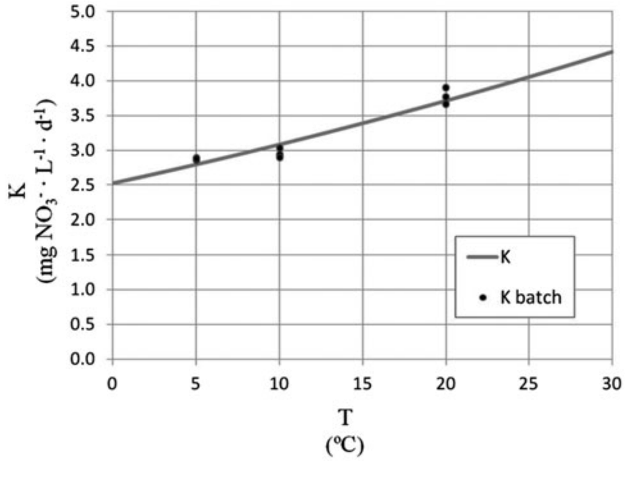

Fig. 2. Experimental overall rate constants found and extrapolation with Arrhenius.

Denitrification was the main cause of nitrate consumption as can be seen in the total nitrogen depletion: 79.7% at 20°C, 84.1% at 10°C, and 82.9% at 5°C with respect to the initial levels (Fig. 3). Ammonium and nitrite remained at lower concentrations, their total, accounting for less than 20% of the total nitrogen at the conclusion of the experiments.

Table 4
Activation energies and transference regimes found in published papers

Bacterial context	Transference regime	References	E_A ($kJ\,mol^{-1}$)
Surface water	Stirred	[23]	46
Deutschland soil	Stirred	[24]	62
Finland soil	Stirred	[24]	50
Sweden soil	Stirred	[24]	57
Clay soils	Column	[25]	50
Sandy loam soil	Column	[25]	55
Lake sediments	Shaken	[26]	77
Hyporheic sediments	Column	[27]	82

3.3. Drought period

This phase caused variations in the final nitrogen distribution compared to temperature variation experimental lines (Fig. 3). Remarkably, a higher nitrate conversion to nitrite with a final nitrogen fraction of 20.0% and ammonium of 33.2% was detected instead of a predominant denitrification process observed in experiments at different temperatures. Only 41.0% of the total nitrogen was eliminated. In addition, the nitrate depletion ratio was sharply increased. Ammonium generation was attributed to DNRA that was presumably favored by the drought conditions. The increase in the final nitrite concentration could be either the result of an intermediate product of denitrification or DNRA processes.

3.4. Freeze-thaw cycle

Nitrogen in the form of nitrate was efficiently consumed reaching a residual level of 4.4% but similarly, to the drought period, this event decreased the conversion of nitrate to nitrogen gas; increasing nitrite until there was a final nitrogen fraction of 18.2% and 20.4% of ammonium. Again, DNRA is the most feasible process for ammonium production and nitrite

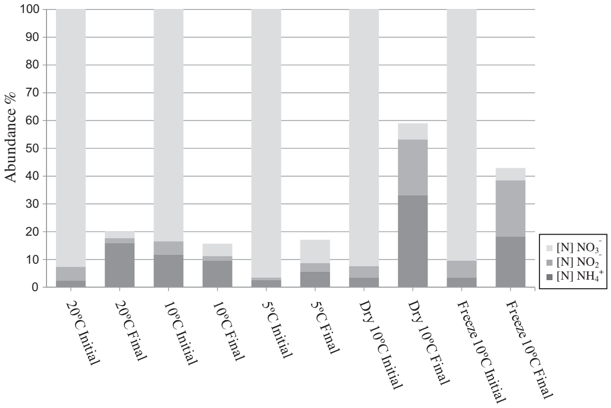

Fig. 3. Relative aqueous nitrogen abundances at initial and final point of the experiments, all species were normalized to nitrogen.

accumulation that could be indistinctly denitrification or DNRA (Fig. 3). In the same way, that during the drought period, nitrate depletion ratio increased.

4. Conclusions

(1) Nitrate has been eliminated in all experiments; with high percentages of above 90%, being mainly as a result of heterotrophic denitrification but shared with DNRA in dried soil and frosted soil. It is reflected in a total nitrogen depletion of over 80% for the experiments at different temperatures and only under 60% in dried soil and frosted soil.

(2) In the study of the variation of the denitrification potential at 20, 10, and 5˚C, the denitrification rate was directly correlated to the temperature. Activation energy was lower in these experiments than in stirred, column, or shaken systems, this behavior is suspected to be due to a predominant restriction in mass transfer (diffusive transport) instead of denitrification kinetic.

(3) Drying and freezing of the soil favors DNRA. Furthermore, in both conditions, nitrite increased sharply and was also accumulated possibly as a DNRA or denitrification intermediate. As a consequence, wetlands permanently flooded with low freezing periods are supposed to exhibit better denitrification potential.

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