Soil Biology and Biochemistry 119 (2018) 203–209

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# Nitrite kinetics during anoxia: The role of abiotic reactions versus microbial reduction

Natalie Y.N. Lim, Åsa Frostegård, Lars R. Bakken*

*Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, 1432, Ås, Norway*

ARTICLE INFO

*Keywords:*
Nitrite kinetics
Chemodenitrification
Acidic soil
Denitrification

ABSTRACT

Anoxic spells in soil induce denitrification, i.e. the sequential reduction $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, catalysed by the four enzymes NAR, NIR, NOR and NOS, respectively. Transient accumulation of all intermediates is inevitable, but the concentrations depend on the regulation of gene expression and the physical/chemical properties of the soil. Nitrite is chemically unstable at low pH, decomposing via a conglomerate of abiotic reactions with metals and organic compounds which can result in production of NO, $N_2O$, $N_2$ and nitrosated organic compounds (R-NO). There is evidence that acidic soils accumulate less nitrite than neutral soils, but it is unclear if this is due to high abiotic decomposition rate ($V_{ADEC}$) or fast enzymatic reduction of nitrite ($V_{NIR}$) at low pH. To investigate this, we monitored the kinetics of $NO_2^-$, NO, $N_2O$ and $N_2$ during anoxic incubations of three organic soils with $pH_{CaCl2}$ ranging from 3.4 to 7.2, taken from a long-term liming experiment. In parallel, we determined the rate of abiotic nitrite decay ($V_{ADEC}$) and its product stoichiometry (NO, $N_2O$ and R-NO) in gamma-irradiated soils. $V_{ADEC}$ was clearly first-order with respect to $HNO_2$ ($k_{HNO2} = 1.4\ h^{-1}$), N-gas production (NO, $N_2O$ and $N_2$) accounted for only ~50% of $V_{ADEC}$, the rest was ascribed to nitrosation (R-NO). During denitrification (live soil incubation), the nitrite concentrations reached 2–3 mM in the soils with pH 4.9 and 7.2, while the soil with pH 3.4 kept nitrite concentrations at 20–50 μM, except for a short spike reaching 160 μM after 40 h. Estimated rates of nitrite scavenging by the two competing sinks (NIR and ADEC) showed that NIR was the strongst nitrite sink in soil with pH 3.4 ($V_{NIR} > V_{ADEC}$), while $V_{NIR} \approx V_{ADEC}$ in the soil with pH 5.9. In the soil with pH 7.2, $V_{ADEC}$ was insignificant. Thus, the regulation of denitrification (high $V_{NIR}$ relative to $V_{NAR}$) played a crucial role in determining nitrite kinetics, hence the fate of nitrite in acid soils. High nitrite reductase activity effectively minimized abiotic nitrite decomposition and nitrosation of soil organic matter. The results shed light on regulation of denitrification in acid soils, and its implications for the fate of nitrogen during denitrification events.

## 1. Introduction

Nitrite is a free intermediate in a number of reactions in the nitrogen cycle, including nitrification, denitrification, and dissimilatory nitrate reduction to ammonium [DNRA, also known as respiratory ammonification (Mania et al., 2014; Yoon et al., 2015)]. It is also an important component of the regulatory networks in these metabolic pathways.

While nitrite is relatively stable and only moderately toxic at high pH, its decomposition, reactivity and toxicity escalates with decreasing pH (Bancroft et al., 1979; Van Cleemput and Samater, 1996), reflecting that $HNO_2$ is more reactive than $NO_2^-$ ($pK_a = 3.3$ for $NO_2^- + H^+ \leftrightarrow HNO_2$), and that cell membranes are permeable to $HNO_2$ but not to $NO_2^-$ (Kaiser and Heber, 1983; Samouilov et al., 2007). Metals can catalyse the reduction of nitrite to NO, $N_2O$ and even $N_2$ (Zhu-Barker et al., 2015). Moreover, $HNO_2$ can react with various organic compounds (nitrosation and nitrosylation; Spott et al., 2011; Heil et al., 2016). Similar reactions have been proposed for nitrate, but this appear to be an experimental artefact (Colman et al., 2007). Finally, nitrite in soils may escape to the atmosphere as gaseous nitrous acid (HONO), and this emission plays an important role in OH formation and tropospheric chemistry (Jacob, 2000; Kulmala and Petäjä, 2011; Su et al., 2011).

Most research on nitrite in soils has focused on the transient accumulation induced by fertilisation with reduced N (urea or ammonium), caused by faster oxidation of ammonia to nitrite than oxidation of nitrite to nitrate. This process is exacerbated by high pH, because nitrite oxidising bacteria are sensitive to $NH_3$ (Ventera et al., 2015; Breuillin-Sessons et al., 2017 Shen et al., 2003). However, nitrite has also been observed to accumulate transiently in soil during denitrification (Glass and Silverstein, 1998; Stevens et al., 1998), and peak concentrations

* Corresponding author.
*E-mail address:* lars.bakken@nmbu.no (L.R. Bakken).

https://doi.org/10.1016/j.soilbio.2018.01.006
Received 1 November 2017; Received in revised form 8 January 2018; Accepted 10 January 2018
Available online 14 February 2018

appear to increase with soil pH, for reasons that are unclear (Shen et al., 2003). It could be due to fast abiotic nitrite decomposition at low pH, but the enzyme kinetics of denitrification could also play a role: early and strong expression of the genes coding for nitrite reductase (*nir*) compared to those coding for $NO_3^-$ reductase (*nar*) in acid soils, would result in marginal accumulation of nitrite. Denitrifying organisms display various regulatory phenotypes regarding the sequence of expression of *nar* versus *nir* (and *nor*, coding for nitric oxide reductase): some organisms reduce all nitrate to nitrite before expressing *nir* and *nor*, others accumulate some nitrite before reducing it further, and yet others express *nar* and *nir* at the same time and therefore display low nitrite accumulation (Liu et al., 2013; Lycus et al., 2017).

The aim of our investigation was to assess the relative importance of abiotic nitrite decomposition versus the enzymatic nitrite reduction during anoxic spells, as influenced by soil pH. We monitored the kinetics of nitrite and N-gases (NO, $N_2O$, $N_2$) during anaerobic incubations of soils of different pH, taken from a long-term field experiment where organic soil was limed to different pH levels. Soils from this field experiment were used in two previous studies of denitrification product stoichiometry (Liu et al., 2010) and for isolating denitrifying organisms (Lycus et al., 2017). We found the expected pH-dependency of nitrite accumulation in soils from this experimental site, i.e., transient nitrite accumulation decreased with pH. To assess the role of abiotic decomposition for the observed nitrite kinetics, we determined the concentration dependent rates of abiotic nitrite decomposition (and the fraction emitted as NO and $N_2O$) by incubating sterilised soils amended with nitrite. The first order decay kinetics, and the product stoichiometry of this decay was used to assess the abiotic versus enzymatic reduction of N species observed in the live soil. This approach allowed an estimation of the relative strength of the two sinks for nitrite, i.e. abiotic decomposition and enzymatic reduction to NO.

## 2. Materials & methods

### 2.1. Soils

Organic soils were collected from a long-term experimental field site in Fjaler, western Norway (61°17′42″N, 5°03′03″E). The site is divided into 24 plots and limed with shell sand, 0–800 $m^3$ per hectare (1977) creating a pH range from pH 3.1 to pH 7.8 (Sognnes et al., 2006). The field experiment has been under permanent grassland since established. In this study, three lime treatments were used: soil **L** (un-limed soil, pH 3.16–3.80), soil **M** (medium lime; 200 $m^3$ shell sand per hectare, pH 5.79–5.89), and soil **H** (high lime; 800 $m^3$ shell sand per hectare, pH 6.77–6.80). Soils from this field experiment were used previously to determine the effect of soil pH on the denitrification product stoichiometry (Liu et al., 2010), and for isolating representative culturable denitrifying organisms (treatments L and H; Lycus et al., 2017).

Two replicate plots were sampled from treatments L and H; and one plot from treatment M. The soil from each plot was analysed separately. Only one plot was sampled from M because shell sand was unevenly distributed in the replicate plot, resulting in a pH that was too close to soil L for our purposes (the pH at the time of sampling was 4.34). All pH values were measured in 0.01 M $CaCl_2$ [1:5 w/w, soil fresh weight (fw) to 0.01 M $CaCl_2$] prior to using the soil. The soil organic C contents were 49, 45 and 40% of dry weight (dw) in soil L, M and H, respectively. The declining C content with increasing pH was primarily due to the increasing amounts of shell sand added in 1977.

The soils were nearly water saturated when sampled (taken during the rainy season), and were immediately dried to reach a moisture level that allowed sieving (8 mm, followed by 4 mm). Large roots and plant residues were removed during the drying process, and the soils were frequently mixed by hand to avoid edge effects. The sieved soils were stored moist [61, 59 and 46% moisture (w/w) in soil L, M and H, respectively] at 4 °C until use. The water holding capacity (WHC) of each soil was determined by flooding and free drainage in filter funnels; WHC was 82, 78 and 68% moisture (% of fw) for soil L, M and H, respectively.

### 2.2. Soil sterilisation

Soil samples were sterilised by gamma-irradiation, to determine the abiotic kinetics of $NO_2^-$ decay and the product stoichiometry of this process. The choice of gamma sterilisation, rather than autoclaving was based on a comparison of gamma sterilisation, chloroform fumigation and autoclaving as to their elimination of biological activity and effects on abiotic $NO_2^-$ decay to NO (described in: Supplementary material S1: Comparison of sterilisation methods).

The soils were given a dose of 27.8 kGy ($^{60}Co$) at the Institute of Energy Technology, Kjeller, Norway. The gamma-irradiated soil was stored for 3 months at 4 °C before use, to deplete free radicals generated by radiolysis.

### 2.3. Nitrite measurements

To monitor the fast degradation of nitrite in the acidic soil, a quick method for measuring nitrate and nitrite was developed. Briefly, 0.2–0.5 g of soil (fw) was transferred to pre-weighed microcentrifuge tubes for nitrite measurement, and sterile MilliQ water (1:2 w/w, soil fw to water) was added to extract the nitrite from the soil matrix. The soil slurry was agitated with a vortex mixer for 5–10 s, then the soil solids were pelleted by centrifugation (17 600 × *g* for 2 min). Then 10 μL of the supernatant was immediately injected into a purging device where nitrite or nitrate + nitrite (depending on reducing agent and temperature) was instantaneously reduced to NO which was transported (by a stream of $N_2$) through a Sievers Nitric Oxide Analyzer 280i system (NOA, GE Analytical Instruments). The integrated NO peaks were used to estimate nitrite and nitrite + nitrate in the injected sample (calibrated by injecting standards). The reducing agents and temperatures were 1 M HCl with ≈50 mM $VCl_3$ (95 °C) to reduce nitrite + nitrate, and 1% w/v NaI in 50% acetic acid (room temperature) to reduce only nitrite. This chemiluminescence nitrate and nitrite measurement is capable of detecting picomole quantities in the injected liquid (Braman and Hendrix, 1989; Cox, 1980).

We suspected that the fast extraction with water could be affected by anion exchange, and tested this by comparing our water extraction procedure with the standard extraction in 2 mM KCl. This comparison was done for nitrate, rather than nitrite, since KCl is suspected to cause degradation of nitrite under acidic and neutral pH conditions (Homyak et al., 2015). The amount of nitrate extracted in water was 50–60% of that extracted by 2 mM KCl (Supplementary Table S1), thus confirming a significant anion exchange capacity of the soils, leading to the recovery of only 50–60% of the nitrate when using our rapid water extraction procedure.

To determine the kinetics of anion exchange, we measured the recovery of nitrite added to gamma-irradiated soils in short-term experiments. Microcentrifuge tubes containing 0.2 g soil fw (≈30% dw) were given a dose of 100 nmol $NO_2^-$ (10 μL of 10 mM $KNO_2$), and extracted with water at 1 min intervals during the first 10 min. The measured concentrations showed a rapid decline during the first 5 min in all soils, approaching apparent equilibrium levels (50–60% recovered) after 8–10 min (Supplementary material, Fig. S2). The concentration dependency of this anion partitioning (sorbed/free anions) was tested by adding a range of nitrite concentrations (50–1000 nmol per vial containing 0.2 g soil fw) which was extracted after 10 min. The fraction of nitrite recovered in the water extract (*F*) was practically constant over the entire concentration range for the two soils tested, $F = 0.49$ and 0.65 for L and H, respectively (Supplementary Material Fig. S3). These values were used for correcting the nitrite concentrations as measured in subsequent experiments (assuming an intermediate *F* value of 0.57 for soil M).