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Abiotic reaction of nitrite with dissolved organic carbon? Testing the Ferrous Wheel Hypothesis

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Abstract The Ferrous Wheel Hypothesis (Davidson et al. 2003) postulates the abiotic formation of dissolved organic N (DON) in forest floors, by the fast reaction of NO_2^- with dissolved organic C (DOC). We investigated the abiotic reaction of NO_2^- with dissolved organic matter extracted from six different forest floors under oxic conditions. Solutions differed in DOC concentrations (15–60 mg L^{-1}), NO_2^- concentrations (0, 2, 20 mg NO_2^--N L^{-1}) and DOC/DON ratio (13.4–25.4). Concentrations of added NO_2^- never decreased within 60 min, therefore, no DON formation from added NO_2^- took place in any of the samples. Our results suggest that the reaction of NO_2^- with natural DOC in forest floors is rather unlikely.

Keywords Abiotic nitrite immobilization · Dissolved organic carbon · Dissolved organic nitrogen · Ferrous Wheel Hypothesis

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Introduction

The abiotic reaction of mineral N with soil organic matter (SOM) is defined as abiotic immobilization and is considered to be a major mechanism of N sequestration in soils. For example, Johnson et al. (2000) concluded that abiotic N immobilization accounts for 6–90% of total N immobilization in a variety of soils. Several studies have postulated abiotic N immobilization to explain the fast disappearance of added NO_3^- in soils (Davidson et al. 1991; Berntson and Aber 2000; Dail et al. 2001; Perakis and Hedin 2001; Compton and Boone 2002; Corre et al. 2007; Huygens et al. 2007; Sotta et al. 2008). Fitzhugh et al. (2003) compared NH_4^+, NO_3^- and NO_2^- immobilization in live and $HgCl_2$-sterilized soils and found that the main pathway for immobilization of NO_2^- was abiotic, while this process was of minor importance for NH_4^+ and NO_3^-. However, the added concentrations in their experiment (0.267 µmol NO_2^--N g^{-1} dry soil) caused a 300–1,300 fold increase in the extractable NO_2^- pool when assuming that in northern hardwood forests NO_2^- concentrations usually range between 0.0002 and 0.0008 µmol g^{-1} (Venterea et al. 2003). In soils, NO_2^- concentrations are generally very low, mostly close to detection limits (Venterea et al. 2003; Müller et al. 2006).

Key factors determining the abiotic reaction of NO_2^- with SOM are organic matter content, soil pH, NO_2^- level, and temperature. The lower the pH and the higher the SOM content, NO_2^- level and temperature, the higher the immobilization of NO_2^- by SOM (Führ and Bremner 1964a, b; Nelson and Bremner 1969). Although NO_2^- immobilization also increases with time of incubation, the reaction of NO_2^- with SOM is fast (Fitzhugh et al. 2003), as over 50% of labeled $^{15}NO_2^-$ were recovered in SOM only 15 min following addition.

Another factor that might influence the abiotic NO_2^- immobilization to SOM is the N status of the ecosystem. Johnson et al. (2000) hypothesized, that abiotic N immobilization is less affected by N status than biotic immobilization. Nevertheless, it seems logical that more N can be immobilized by SOM if less binding places for N are occupied.

The Ferrous Wheel Hypothesis (Davidson et al. 2003) postulates the abiotic reaction of NO_2^- with dissolved organic carbon (DOC) instead of SOM, leading to formation of dissolved organic N (DON) and to N sequestration. The hypothesis tries to explain the fast removal of added NO_3^- in forest soils. It implies that NO_3^- in the forest floor of upland soils is first reduced by Fe(II) or Mn(II) to NO_2^-. The recovery of metals occurs as the oxidized metals are reduced again by SOM. The reaction of NO_2^- with DOC was postulated based on the fast reaction of NO_2^- with defined phenolic compounds (e.g. syringic acid, vanillic acid) used as surrogates for natural DOC (Davidson et al. 2003). The reaction of NO_2^- with defined phenolic compounds (α-naphthol) was also reported by Azhar et al. (1989).

The Ferrous Wheel Hypothesis explicitly states that DOC (not solid SOM) is the acceptor for NO_2^-. The advantage of working with dissolved (DOC) instead of solid SOM when investigating abiotic NO_2^- immobilization is obvious. To separate abiotic from biotic reactions, it is necessary to effectively sterilize soils with minimum alteration of soil chemical and physical properties (Wolf and Skipper 1994). By definition, dissolved organic matter (DOM) is sterilized by filtration through 0.2 μm pore size filters to exclude microorganisms. This technique is fast, inexpensive and does not cause any physical or chemical alteration of the sample. The sterilization of the sample by addition of $HgCl_2$, which was suggested for soils (Wolf and Skipper 1994) is not suitable since Hg would change the redox chemistry. Moreover complexation of DOC with Hg will alter the structure of DOC.

To our knowledge, the abiotic reaction of NO_2^- with natural DOC has not been shown until now. Our aim was therefore to test whether an abiotic reaction of NO_2^- with natural DOC leads to fast DON formation at reasonable DOC and NO_2^- concentrations.

Materials and methods

Samples were collected from the Oi and the Oa horizon of three long-term ecosystem research sites: the Chi-Lan Mountain forest ecosystem in Northern Taiwan (Cypress, *Chamaecyparis formosana* var. *obtusa*), the Steinkreuz site (European beech, *Fagus sylvatica* L.), and the Coulissenhieb site (Norway spruce, *Picea abies* (L.) Karst.), both in Germany. Details concerning climate, soils, and vegetation can be found in Rees et al. (2006) and Gerstberger et al. (2004). The soils were chosen to cover different pH, SOM, and DOC qualities (Table 1). Moreover, they

Table 1 Properties of the different forest floor horizons and their water extracts

	pH ($CaCl_2$)	CEC_{eff} ($mmol_c$ kg^{-1} soil)	BS (%)	TOC (g kg^{-1})	TON (g kg^{-1})	TOC/TON of forest floor	DOC/DON of extract	pH
European beech Oi	4.7	567.9	86.7	445	20.4	21.8	15.7	6.3
European beech Oa	3.4	172.3	65.7	205	11.5	17.8	14.5	5.8
Norway spruce Oi	3.6	245.8	22.7	478	19.3	24.8	13.4	5.4
Norway spruce Oa	2.6	274.2	56.8	376	16.6	22.6	15.7	4.4
Cypress Oi	2.8*	83.6*	55.6*	340*	20*	17*	25.4	4.7*
Cypress Oa							24.7	

CEC_{eff} effective cation exchange capacity, *BS* base saturation, *TOC* total organic carbon, *TON* total organic N (Gerstberger et al. 2004; Rees et al. 2006; own data)

* Data only available for a mixed sample of Oi and Oa

represent different N saturation stages, as revealed by the DOC/DON ratios (Table 1) and the fluxes of DON and mineral N in the ecosystem (Table 2).

Dissolved organic C was prepared by adding 3 L of water to 300 g fresh weight of litter. The litter samples were previously frozen. The suspensions were then stored at 5°C and stirred three times. After 24 h the solutions were first filtered by suction through a ceramic plate (pore diameter about 1 µm). To exclude microorganisms, the solutions were then filtered through 0.2 µm cellulose acetate filters (Schleicher and Schüll OE 67), which were prewashed with 2 × 150 mL of pure water. Therefore, sterility of the solutions was assumed for the time of the measurement (60 min).

Table 2 Fluxes of NH_4^+, NO_3^-, DON and DOC in throughfall and in forest floor percolates (Oa) at the three experimental sites

	European beech[a]	Norway spruce[b]	Cypress[c]
Throughfall (kg ha^{-1} year^{-1})			
NH_4^+-N	7.0	9.2	2.5
NO_3^--N	6.2	11.5	1.4
DON	3.9	3.4	2.4
DOC	77	84.1	151.2
Forest floor percolates (Oa) (kg ha^{-1} year^{-1})			
NH_4^+-N	5.1	3.9	2.6
NO_3^--N	14.8	8.4	3.4
DON	6.1	4.7	13.0
DOC	176	114.7	1,137.6

[a] Kalbitz et al. (2007) (years 1999–2005)

[b] Michalzik and Matzner (1999) (years 1995–1997)

Chang et al. (2007) (years 2003–2004), values recalculated from 15 to 12 months

Dissolved organic C concentrations of all solutions were adjusted to 15 and 60 mg C L^{-1}, and NO_2^- was added in concentrations of 2 or 20 mg N L^{-1} as $NaNO_2$ at room temperature (22°C). If original DOC concentrations in the soil extracts were too low, solutions were freeze-dried and adjusted with distilled water to the desired concentration (for 15 mg C L^{-1}: Cypress Oi, Cypress Oa; for 60 mg C L^{-1}: Cypress Oi, Cypress Oa, Norway spruce Oi, Norway spruce Oa). Each treatment had three replicates. Nitrite concentrations were determined after 0, 15, 30, 45 and 60 min with a segmented flow analyzer (Skalar San^{plus} Analyzer) at 540 nm as highly coloured azo dye which is formed as NO_2^- is diazotized with sulfanilamide and coupled with α-naphthylethylenediamine dihydrochloride.

Results and discussion

During 60 min of reaction time, NO_2^- concentrations did not change in any of the DOM samples independent of DOC and NO_2^- concentrations (Fig. 1, data exemplarily shown for Norway spruce Oi and Oa with 60 mg C L^{-1}, other data not shown). Hence, a formation of DON by a fast, abiotic reaction of NO_2^- with DOM can be ruled out. Even if solutions were not totally sterile, microbes potentially present in solutions did not influence NO_2^- concentrations.

The fact that no abiotic reaction took place can be attributed to several factors. First, DOC concentrations might have been too small, as abiotic NO_2^- immobilization in soils was shown to increase with solid SOM concentrations (Nelson and Bremner 1969; Führ and Bremner 1964a, b). However, in our study even a fourfold increase from 15 to 60 mg DOC L^{-1} did not trigger NO_2^- immobilization. Such

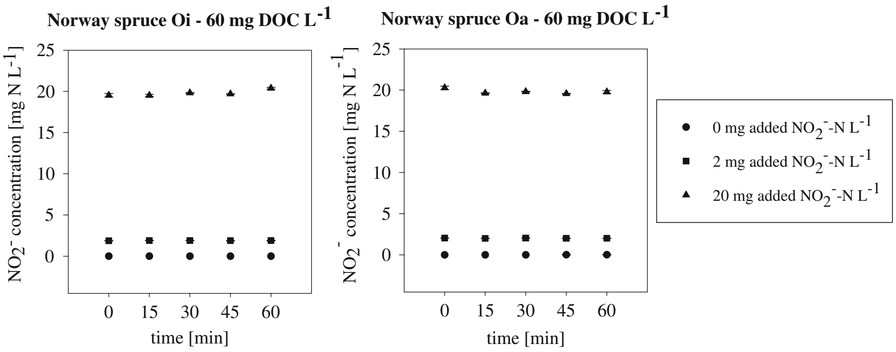

Fig. 1 Temporal course of NO_2^- concentrations in water extracts from forest floors. *Error bars* are ±1 SE of the mean and $n = 3$

DOC concentrations are commonly found in forest floor percolates under field conditions (Michalzik et al. 2001).

Second, NO_2^- concentrations might have been too small as the immobilization of NO_2^- in soils increased with increasing NO_2^- concentrations (Nelson and Bremner 1969; Führ and Bremner 1964a). But even at very high concentrations of NO_2^- (20 mg N L^{-1}), no reaction took place. As NO_2^- concentrations in soils are usually much less (Venterea et al. 2003; Müller et al. 2006), we do not expect a reaction to take place under field conditions.

It has been shown that the immobilization of NO_2^- by SOM increases with increasing temperature (Nelson and Bremner 1969). The temperature of the solutions (22°C) in our experiment exceeded the average soil temperatures commonly found in temperate forest soils by far. Hence, the reaction of NO_2^- under field conditions appears to be unlikely if no reaction occurs under higher temperatures in the laboratory.

Our solutions differed in DOC/DON ratios but, although we expected to find differences in the reaction of NO_2^- with DOC, none were observed. Also the N status of the ecosystem had no influence considering the variation of N fluxes in throughfall and forest floor percolates at the sites. Nitrite immobilization was found to be highest in acid soils (Nelson and Bremner 1969), however, the differences in pH (4.4–6.3) did not lead to differences in NO_2^- immobilization in our study.

The reaction time might have been too short, but Davidson et al. (2003) showed that NO_2^- as highly reactive to defined phenolic compounds at pH 4 with significant decreases of added NO_2^- concentrations after 30 min. The authors used NO_2^- concentrations of 14 mg NO_2^--N L^{-1} and equimolar additions of phenols (12 mg C L^{-1}). Although in our study, NO_2^- concentrations (0, 2, 20 mg NO_2^--N L^{-1}) and DOC concentrations (15 and 60 mg C L^{-1}) were very similar or higher, no reaction took place. Dissolved organic carbon is mostly comprised of humic substances with a much more complex chemical structure and higher N contents as compared to defined phenols. The DOC used in this experiment had aromatic C contents ranging only from 9 to 30% (Scheel et al. 2007). Thorn and Mikita (2000) demonstrated nitrosation reactions of humic substances with NO_2^- by NMR, but they used very high NO_2^- concentrations that do not occur in soils. In one case they showed nitrosation reactions at low NO_2^- concentrations but then the reaction time was very long (9 days) which does not support the idea of a fast reaction of NO_2^- with humic substances. Their approach also did not include a mass balance for added NO_2^- and a quantitative analysis of NO_2^- immobilization.

Nevertheless, a fast removal of added NO_3^- in sterilized soils has often been observed (e.g. Dail et al. 2001; Fitzhugh et al. 2003). Recently, Colman et al. (2007) challenged the observations on NO_3^- disappearance made by Dail et al. (2001), and pointed to possible iron interference during colorimetric determination of NO_3^- and subsequent underestimation of NO_3^- concentrations. This in turn would lead to an overestimation of abiotic NO_3^- immobilization. Davidson et al. (2008) tested the possible iron interference in the samples used by Dail et al. (2001) and concluded that iron concentrations were too low to lead to analytical errors.

Colman et al. (2007) might have failed to repeat the findings of Dail et al. (2001) because they likely destroyed anaerobic microsites in their samples (Davidson et al. 2008), which allow NO_3^- reduction to NO_2^-. In our study, NO_2^- was already available and the reduction of NO_3^- was not a limiting factor. However, anaerobic microsites could influence the redox status of soil organic matter which in turn could affect NO_2^- immobilization. It has been shown, that humic substances are redox-active (e.g. Bauer et al. 2007; Heitmann et al. 2007), but the effect of the redox status of DOM on reactions with reduced N compounds is unknown.

For explaining the fast disappearance of added NO_3^- other intermediates of NO_3^- reduction or radicals might be considered as reactants. Another option is that the reaction requires the presence of a solid phase, such as solid organic matter or even a mineral surface as catalyst. Some observations of abiotic NO_3^- immobilization might also be due to artefacts, e.g. changes in SOM quality induced by sterilization or incomplete sterilization in general (lack of homogenization of sterilant and soil and subsequent formation of unsterile microsites).

According to our study, the postulated third step of the Ferrous Wheel Hypothesis, (reaction of NO_2^- with DOC) is not likely to occur under realistic concentrations of the reactants and under the experimental conditions used. Therefore, we conclude that the Ferrous Wheel Hypothesis needs revision and that other mechanisms of abiotic NO_3^- immobilization should be investigated.

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