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Inhibitory and side effects of acetylene (C_2H_2) and sodium chlorate ($NaClO_3$) on gross nitrification, gross ammonification and soil-atmosphere exchange of N_2O and CH_4 in acidic to neutral montane grassland soil

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ABSTRACT

Nitrification is a central component of the terrestrial nitrogen (N) cycle, but the contribution of autotrophic and heterotrophic nitrification to total gross nitrification remains poorly understood. To clarify their relative importance in neutral and moderate acid soils, an incubation experiment was conducted with ^{15}N-ammonium isotopic pool dilution techniques and combined with acetylene (C_2H_2, 10 Pa) as a specific inhibitor of autotrophic nitrification and sodium chlorate ($NaClO_3$) as a potential inhibitor of heterotrophic nitrification. Additionally, CO_2, N_2O and CH_4 fluxes were measured to identify potential side-effects of inhibitors on soil respiration and CH_4 fluxes.

The presence of C_2H_2 completely eliminated gross nitrification in all investigated soil samples. The addition of $NaClO_3$ affected neither gross nitrification nor gross ammonification in soils of both investigated grassland sites. This provided strong evidence that heterotrophic nitrification was not an important process in the investigated grassland soils. Acetylene but not $NaClO_3$ decreased net CH_4 uptake, likely due to homology of the enzymes ammonia monooxygenase. Overall, the present study shows a dominant role of autotrophic nitrification in gross nitrate production for both neutral and slightly acid soils and illustrates the potential of acetylene as an inhibitor of gross autotrophic nitrification.

1. Introduction

Nitrification, the microbial oxidation of ammonia (NH_3) to nitrite (NO_2^-) or further to nitrate (NO_3^-), plays a key role for nitrogen (N) cycling in terrestrial ecosystems [1] by affecting major ecological processes such as net primary productivity [2–4] and net ecosystem exchange [5,6] as well as ecosystem N losses via leaching [7,8] and gaseous pathways, e.g. via the potent greenhouse gas nitrous oxide (N_2O) [9,10]. Nitrous oxide is either directly produced by nitrification or by subsequent denitrification [11]. Nitrification is performed by both autotrophic and heterotrophic microorganisms with pH playing an important role in the regulation of the importance of autotrophic vs. heterotrophic nitrification. Heterotrophic nitrification has been assumed to be of importance only in acidic soils [12–15]. In contrast, for soils with neutral pH values, it has been assumed for decades that autotrophic nitrification is the sole process with no contribution of heterotrophic nitrification [16–18]. However, these studies were usually based on methodologies with constraints such as the determination of net nitrification rate [19] or culture studies [12,20], i.e., methods not providing actual gross nitrification rates.

Improved understanding of nitrification has been achieved by the application of the ^{15}N pool dilution technique which provides gross rather than net rates of nitrification [21–23]. However, due to methodological difficulties, the separation of total gross NO_3^- production into process-specific pathways, i.e. gross autotrophic nitrification (oxidation of free soil ammonium) and gross heterotrophic nitrification (also involving direct oxidation of organic N compounds to NO_3^-) [4,24] has seldom been studied [12,13,25].

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Specific inhibitors such as C_2H_2 at low concentrations (10 Pa) have been used to inhibit the NH_3 oxidation by the activity of autotrophic nitrifiers [1,26]. To current knowledge heterotrophic nitrification does not rely on the enzyme NH_3 monooxygenase and thus, is not inhibited by common nitrification inhibitors such as C_2H_2 or nitrapyrin [26]. However, chlorate was found to inhibit heterotrophic nitrification in acid forest humus [16], which was confirmed by Lang (1986), who found that chlorate blocks nitrification in acid forest soil from the Solling site, i.e. a site at which autotrophic nitrifiers could not be detected. On the other hand, several studies reported that $NaClO_3$ also inhibits ammonia monooxygenase [17,19]. Such inhibitors have often been used in combination with indirect parameters of soil N turnover such as microbial biomass, net nitrification, enzyme activity and C and N gas fluxes, culture studies [12,17,20,24,27,28], but have rarely been linked with studies determining gross rates of N turnover and simultaneous determination of soil-atmosphere exchange of N_2O, CH_4 and CO_2 [29]. Indeed very few studies investigated the role of autotrophic vs. heterotrophic gross nitrification for soils with slightly acidic or neutral pH values [30]. Therefore, inhibitor effects on total gross nitrification as well as the quantitative contribution of autotrophic versus heterotrophic nitrification to gross NO_3^- production and N_2O formation are actually much more uncertain than is suggested by abundant studies using traditional indirect methods for the characterization of nitrification. Furthermore, inhibitor side effects on CH_4 and CO_2 are uncertain [31].

Recently the application of numerical ^{15}N tracing models [4,32] and studies using analytical $^{15}NH_4^+$ and $^{15}NO_3^-$ pool dilution approaches [32] indicated a significant contribution of the direct conversion of organic N to nitrate, ascertained to heterotrophic nitrification. This challenged the previous views of the absolute dominance of autotrophic nitrification in slightly acidic or neutral soils. In order to contribute to the clarification of this issue, we combined ^{15}N pool dilution approaches to quantify gross nitrification and the selective inhibitor C_2H_2 to investigate the role of heterotrophic versus autotrophic nitrification in slightly acidic and neutral grassland soils of Southern Germany. We also included a $NaClO_3$ treatment to compare C_2H_2 vs. $NaClO_3$ effects on gross nitrification with the aim to contribute to understanding of the contradictory results of $NaClO_3$ effects on either autotrophic or heterotrophic nitrification in earlier studies.

Inhibitor treatments and measurements of gross N turnover were accompanied by measurements of soil-headspace exchange of CO_2, CH_4 and N_2O to evaluate the importance of heterotrophic vs. autotrophic nitrification in the production of N_2O at different pH levels and to identify potential side-effects of inhibitors on soil respiration and CH_4 fluxes.

2. Material and methods

2.1. Soil sampling and measurement of soil properties

Soil samples were collected at two typical pre-alpine grassland ecosystems of Southern Germany (Graswang site: 11.03°E; 47.57°N; and Wielenbach site: 11.15°E, 47.89°N) with calcareous soil and pH values of 7.33 and 5.94 (Table 1). Both sites are located in the flood plain of the Ammer river catchment. The Graswang site is located in the upper part of the catchment at 865 m a.s.l., surrounded by calcareous alpine mountain ranges, while the Wielenbach site is located downstream in the lower pre-alpine part of the catchment at 545 m a.s.l. This altitudinal gradient induces a climatic gradient: the mean annual temperature at the Graswang site is 6.0 °C and the mean annual precipitation is 1437.6 mm, while at the Wielenbach site, the mean annual temperature is 7.8 °C and the mean annual precipitation is 1020.5 mm during 50 years (1955–2005).

Table 1
Topsoil (0–10 cm soil depth) characteristics of grasslands in Graswang and Wielenbach ($n = 3$).

$N \geq 3$	Graswang	Wielenbach
Soil total organic carbon (%)	13.6 ± 0.3	6.8 ± 1.1
Soil total nitrogen (%)	0.81 ± 0.04	0.68 ± 0.10
C:N	16.9 ± 0.5	10.0 ± 0.2
Soil pH value	7.33 ± 0.08	5.94 ± 0.01
Soil bulk density (mg cm^{-3})	0.98 ± 0.03	1.08 ± 0.04
Soil nitrate concentration (mg kg^{-1} SDW)	19.1 ± 3.9	41.4 ± 1.9
Soil ammonium concentration (mg kg^{-1} SDW)	0.29 ± 0.01	0.25 ± 0.04

Soil samples of the Ah horizon (0–10 cm) were collected in March 2010. Soil was sampled randomly at three locations at each site as intact surface soil of approximately 3 kg each (Fig. 1). Following immediate transfer to the laboratory of IMK-IFU, soil was pooled within sites, air dried and sieved for removal of stones >5 mm, roots >1 cm and soil macrofauna such as earthworms. Subsequently soil was stored at 4 °C until further processing. All experiments were carried out in the laboratory.

2.2. Laboratory incubation

Three different incubation treatments were performed: a) control, i.e. no addition of inhibitors; b) addition of C_2H_2 (10 Pa) to the headspace to inhibit autotrophic nitrification and, c) addition of $NaClO_3$ (100 mg per kg soil dry weight). This general experimental set-up was repeated three times (Fig. 1).

Three days prior to experiments all soil samples were re-wetted to 65% maximum water holding capacity by adding the respective amount of standard rain solution [33]. Following the pre-incubation period of three days a subsample was taken for the determination of soil NH_4^+ and NO_3^- concentrations as well as microbial biomass. Thereafter, the soil was split into three subsamples (treatments: control, C_2H_2, $NaClO_3$), and these subsamples were further divided into two subsamples for determination of gross ammonification and gross nitrification rates. Subsamples used to quantify gross N turnover were further subdivided into six subsamples of 30 g each, with three subsamples each being used for the first and second extraction after isotope labeling to quantify gross N turnover (see below) (Fig. 1).

2.3. Determination of gross rates of ammonification and nitrification

Gross rates of ammonification and nitrification were determined by the ^{15}N pool dilution method [34]. For this purpose, soil was labeled with either ^{15}N–$(NH_4)_2SO_4$ or ^{15}N–KNO_3^- solution at 50 atom% ^{15}N enrichment and an application rate of 3 ml 100 g^{-1} dry soil equivalent. The ^{15}N solution was sprayed on soil in five steps with subsequent mixing to ensure homogenous labeling. The amount of added N corresponded to 2 mg N kg^{-1} soil. Hence, we increased soil NH_4^+ availability by a factor of approximately five in soil samples used for the determination of gross ammonification rates (see Table 1 for background NH_4^+ and NO_3^- concentrations). However, adding 2 mg of NO_3^-–N to the soil for determination of gross nitrification did alter soil NO_3^- concentrations only marginally (factor 1:10 for Graswang and factor 1:20 for Wielenbach, respectively). Thirty grams of labeled soil was filled into glass flasks (338 ml volume), which were immediately closed gas-tight with rubber stoppers. Inhibitors were added either with the labeling solution (in case of $NaClO_3$) or by injecting C_2H_2 to the headspace of the gas-tightly closed incubation vessels so that an end concentration of 10 Pa was achieved.