Agriculture, Ecosystems and Environment 141 (2011) 230–233

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

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

Short communication

Proximal and distal control by pH of denitrification rate in a pasture soil

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ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form 9 February 2011
Accepted 11 February 2011
Available online 5 March 2011

Keywords:
Denitrification
pH
Nitrous oxide
Dinitrogen
Denitrifying enzyme activity
Soil

ABSTRACT

Soil pH can influence denitrification both proximally and distally. Proximal control by pH involves direct changes in denitrification reductase activity while distal control by pH involves changes in the denitrifier community, which is a key component affecting the denitrification rate. The current study separated the proximal and distal control by pH of the denitrification rate and of the relative proportion of two denitrification gas products (N_2O and N_2). The potential denitrifying enzyme activity (DEA) was measured in the presence or absence of acetylene in three pasture soils differing in pH management in the field. The pH of these soils was further manipulated just before DEA measurement to determine the effect of short-term changes in pH. DEA was driven by the pH management in the field rather than by current pH resulting from short-term changes in pH. However, the $N_2O/(N_2O + N_2)$ ratio was driven by the effects of the current pH value on the kinetics of N_2O production and reduction. The data suggest that even if the pH-induced changes in the structure of denitrifying community can control the absolute denitrification rate (distal control by pH), the community does not influence the proportion of denitrification products, which is regulated solely by the proximal control by pH.

1. Introduction

In conjunction with nitrification, denitrification is the main soil process responsible for emissions of nitrous oxide (N_2O) (Conrad, 1996), a significant contributor to stratospheric ozone depletion and global warming (Conrad, 1996; Knowles, 1982). Soil pH is a crucial abiotic factor influencing not only the denitrification rate but, even more importantly, the proportion of the two major denitrification products, N_2O and N_2 (Šimek and Cooper, 2002). In general, activity of denitrification enzymes increases with increasing pH values (up to the pH optimum), while in contrast the $N_2O/(N_2O + N_2)$ ratio decreases. Wallenstein et al. (2006) defined the effects of environmental parameters (including pH) on the kinetics of denitrification enzymes as "proximal control". Soil pH, however, also influences the denitrifier community (Enwall et al., 2005; Parkin et al., 1985), whose abundance and/or composition can be important drivers of denitrification activity and molar ratio of denitrification products (Bremer et al., 2009; Cavigelli and Robertson, 2000; Holtan-Hartwig et al., 2000). Wallenstein et al. (2006) defined this effect as "distal control" because it influences the composition and abundance of the denitrifying community over the long term. The denitrifying community, in turn, acts as a transducer through which proximal controls on denitrification are realized. The concept of "proximal and distal control" is very useful, because even if both controls stabilize during different time periods (short- and long-term, respectively), in the end they both influence the instantaneous activity of denitrification enzymes.

In our previous study (Čuhel et al., 2010), we showed that a 10-month manipulation of soil pH in an experimental grassland led to changes in the abundance of denitrifiers possessing the *nirS* gene encoding cytochrome cd_1 nitrite reductase (NirS). The previous study also documented a relationship between the abundance of NirS-denitrifiers and denitrifying enzyme activity (DEA). It was not clear, however, whether soil pH influenced denitrification rate directly through the kinetics of the denitrification reactions (proximal pH control) or indirectly through the size of denitrifying community possessing this gene (distal pH control). The objective of the present study was to explore how soil pH influences the denitrification rate and $N_2O/(N_2O + N_2)$ ratio both proximally (direct pH effect) and distally (indirect pH effect).

2. Materials and methods

Soils were sampled at an experimental field in a grassland area in South Bohemia, Czech Republic, which is described in detail in our previous study (Čuhel et al., 2010). Briefly, the experimental site was established in July 2007 and included 12 plots (each 3 m × 3 m) with three different pH treatments: each of four plots was amended three times (in July 2007, September 2007, and April 2008) with a KOH solution, with an H_2SO_4 solution, or with water as described by Čuhel et al. (2010). Three independent soil samples were taken from each plot (12 samples for each pH treatment) in July 2009.

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doi:10.1016/j.agee.2011.02.016

Table 1
Soil pH, moisture, mineral nitrogen (NH_4^+, NO_3^-) content, total nitrogen (N_{tot}), organic carbon (C_{org}), carbon in microbial biomass (C_{mic}), and glucose-induced respiration (GIR) in soils from the experimental plots differing in pH treatment. Values are means ± standard deviations ($n = 4$). Values in a row followed by different letters are significantly different ($P < 0.05$).

Parameter[A]	Soil		
	Acidic	pH-Natural	Alkaline
pH (H_2O)	5.00^a ± 0.02	6.03^b ± 0.01	7.07^c ± 0.04
Moisture (g H_2O g^{-1})	0.207^a ± 0.004	0.206^a ± 0.004	0.195^b ± 0.003
NH_4^+ (μg N g^{-1})	0.82^a ± 0.00	0.55^b ± 0.08	0.99^c ± 0.08
NO_3^- (μg N g^{-1})	2.79^a ± 0.16	13.93^b ± 0.16	15.06^c ± 0.28
N_{tot} (mg N g^{-1})	3.82^a ± 0.19	1.30^b ± 0.26	2.83^{ab} ± 1.59
C_{org} (mg C g^{-1})	23.1^{ab} ± 1.3	21.0^a ± 0.6	24.2^b ± 1.1
C_{mic} (μg C g^{-1})	308.3^a ± 44.6	397.3^a ± 75.0	675.3^b ± 64.0
GIR (μg C g^{-1} h^{-1})	10.5^a ± 0.4	21.1^b ± 1.4	24.5^c ± 1.9

[A] For details on methods, see Čuhel et al. (2010) and references listed ibid.

The samples were passed through a 5-mm mesh sieve, combined to produce one composite sample for each pH treatment, and stored at 4 °C in plastic bags. The soil samples were labelled acidic, pH-natural, and alkaline (Table 1).

DEA was measured during the first 7 days after soil sampling, while less sensitive soil characteristics like total N and organic C (Table 1) were analyzed later, in the following 2 weeks. DEA was determined in the soils by the phase I assay of Smith and Tiedje (1979), which was slightly altered as described in Šimek and Hopkins (1999) and in this paper. Soil was placed in 120-ml serum bottles (four replicate bottles per soil treatment and 25 g of field-moist soil per bottle) and allowed to equilibrate to 25 °C for 1 h. Then 20 ml of water, H_2SO_4, or KOH solution (see next paragraph) was added to the bottles, and the resulting slurries were vigorously shaken. After 20 min of equilibration, 5 ml of the glucose solution (1000 mg l^{-1}) and KNO_3 solution (500 mg l^{-1}) used for DEA determination was added, the bottles were vigorously shaken again, and pH of the slurries was measured using a combined electrode (SenTix 61, WTW, Germany) and pH meter (526/538 pH Meter, WTW, Germany). Bottles were capped with rubber stoppers and metal holders and were evacuated and flushed four times with 99.99% He. The slurries were then incubated either with or without acetylene (10%, v/v) on an end-to-end shaker at 25 °C for measurement of DEA ($N_2O + N_2$) and N_2O production. After 30 and 60 min, the concentration of N_2O in the headspace was quantified using gas chromatography (for details see Čuhel et al., 2010); then the pH of the slurries was measured again.

We first determined DEA in the three soils (acidic, pH-natural, and alkaline) sampled in the field without any additional pH manipulation. We then used other portions of the pH-natural soil and added 20 ml of 4.5 mM H_2SO_4 or 8.8 mM KOH solutions to the soil slurries just before DEA measurement to shift their original pH values (6.03) to those of the acidic (pH 5.00) or alkaline (pH 7.07) soils, respectively. Finally, we used the acidic and alkaline soils and shifted their pH values to that of the pH-natural soil just before DEA measurement by adding 20 ml of 9.4 mM KOH or 2.7 mM H_2SO_4 solutions to the soil slurries, respectively. Concentrations of H_2SO_4 and KOH solutions necessary to change pH to target values were determined in preliminary experiments. Results of pH determination before and after DEA measurement showed that adjusted pH values fluctuated less than ±0.2 pH units during 1 h of incubation. Therefore, we calculated the adjusted pH values as the average pH before and after DEA measurement.

The relationships between pH and DEA or the $N_2O/(N_2O + N_2)$ ratio were evaluated by calculating Spearman's rank correlation coefficients, and the differences between the effects of long-term (field manipulation) and short-term (laboratory manipulation) changes in pH on DEA (or the $N_2O/(N_2O + N_2)$ ratio) were analyzed by the Wilcoxon rank sum test. The statistical analyses were performed using the R package version 2.12.1 (R Development Core Team, 2010).

3. Results and discussion

Analysis of DEA in the soils with different field pH management resulted in the previously described pattern (Šimek and Cooper, 2002): in agreement with our previous findings (Čuhel et al., 2010), DEA was highest in the alkaline soil and lowest in the acidic soil (Fig. 1A) but the $N_2O/(N_2O + N_2)$ ratio was highest in the acidic soil and lowest in the alkaline soil (Fig. 1D). Although DEA of the pH-natural soil did not change if the soil pH was decreased by addition of H_2SO_4 just before DEA determination (Fig. 1B), DEA of the pH-natural soil did increase if the soil pH was increased by the addition of KOH just before DEA determination. The difference between the alkaline and pH-natural soil, however, was greater than the difference between the pH-natural soil with and without the addition of KOH (Fig. 1A vs. B). In contrast, pH manipulations of the pH-natural soil shifted the $N_2O/(N_2O + N_2)$ ratios (Fig. 1E) to those found in the soils differing in pH (Fig. 1D); we did not find any difference in the $N_2O/(N_2O + N_2)$ ratio between acidic soil and pH-natural soil adjusted to an acidic pH or between alkaline soil and pH-natural soil adjusted to an alkaline pH (compare Fig. 1D and E). These results are also supported by DEA measurements (Fig. 1C) and $N_2O/(N_2O + N_2)$ ratios (Fig. 1F) of acidic and alkaline soils whose pH values were adjusted to that of pH-natural soil just before DEA determination (after adjustment, the pH of the acidic and alkaline soil was 5.95 and 6.08, respectively). While pH adjustment did not alter the DEA of the acidic and alkaline soils (compare Fig. 1A and C), the $N_2O/(N_2O + N_2)$ ratios were shifted to values typical of the pH-natural soil (Fig. 1F).

The weak correlation between pH and DEA (Spearman's rank correlation coefficient $r = 0.643$; $P = 0.069$) indicated that DEA was dependent not only on the current pH value but also on the "pH history" of the studied soils. Further, the Wilcoxon test revealed that the long-term pH effect was more important for DEA than the short-term pH effect at a risk of 6.7% ($P = 0.067$), which could be considered significant considering the low number of measures. On the other hand, the negative correlation between pH and the $N_2O/(N_2O + N_2)$ ratio (Spearman's rank correlation coefficient $r = -0.964$; $P = 0.001$) showed that the current pH value was extremely important for relative N_2O production. This finding was also supported by the Wilcoxon test ($P = 0.400$), indicating that the pH shift achieved in the laboratory on a short-term basis did not influence the relative N_2O production more than the pH shift achieved by the field adjustment.

Although hundreds of papers published in the last three decades have used DEA measurements for estimating the denitrification rate, there are still some doubts about the relationship between the DEA data and actual denitrification, as recently discussed, e.g., by Oehler et al. (2010). DEA estimates the process of denitrification by incubation under optimal laboratory conditions and is believed to represent the size of the denitrifying enzyme pool present in the soil sample at the time of measurement (Smith and Tiedje, 1979). We found it advantageous to use DEA in a present study because it is a standardized technique in which environmental factors (other than pH) are invariable and strictly defined and could not overshadow the effect of pH. As noted, however, DEA measures potential rather than actual denitrification, and this limitation should be recognized when considering the data reported in this paper.

Our present results clearly indicate that DEA (overall N_2O and N_2 production) was more affected by the relatively long-term pH management in the field (2 years in this case), which led to the changes in the abundance of denitrifiers possessing the *nirS* gene encoding cytochrome cd_1 nitrite reductase (NirS) (Čuhel et al., 2010), than by short-term changes in pH. We expect that, in addition to the changes of denitrifier abundance (Čuhel et al., 2010) and of other soil parameters (Table 1), the pH adjustment in the field also changed the composition of the denitrifying community for the following reasons: soil pH is an important factor driving bacterial community composition (Fierer and Jackson, 2006); 2 years of liming was shown to be sufficient to change the structure of bacterial community in grassland soils (Gray et al., 2003); and the ability to denitrify has been identified in a very diverse group of phylogenetically unrelated bacteria (Zumft, 1997). It is evident that DEA as a measure of the denitrification rate in the present study was controlled by pH indirectly, i.e., denitrification was evidently more affected by the size and composition of denitrifying community than by the current or direct pH effect, even if we did not analyze the composition of denitrifier community in the experimental soils and cannot separate the effects of the community abundance vs. those of community composition. The direct pH effect can also substantially control denitrification rate as shown by Šimek et al. (2002), but the direct effect of pH in the current study was probably limited by the relatively narrow range of pH values. The pH range did, however, support a substantial indirect effect of pH on DEA.

On the other hand, the relative production of N_2O and N_2, here expressed as the $N_2O/(N_2O + N_2)$ ratio, was affected by the current pH value of the soil slurries during measurement rather than by the long-term pH value. Thus, the kinetics of N_2O production and reduction were controlled exclusively by the direct pH effect. When pH is adjusted just before DEA determination, it is unlikely that the denitrifying community can change its abundance or composition, and induction of new