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Nitrogen Mineralization and Nitrification in Two Soils with Different pH Levels

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ABSTRACT

Soil pH is one of the properties that mostly influences nitrification rates, and can be used as a tool for controlling this process, seen that depending on its extent it may lead to nitrogen (N) losses and subsequent contaminations. The aims of this study were to evaluate mineralization and nitrification of two soils at different pH levels. The experimental design was factorial with two factors and three replicates, with the first factor referring to two samples of red latosols, one eutrophic (LV1) and the other dystrophic (LV2), and the second factor was soil's pH, at six levels: 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5. Samples were incubated for 70 days in laboratory conditions. Both nitrate (N-NO_3) and mineral N contents were determined and adjusted to growth models. The eutrophic soil presented higher mineral N and N-NO_3, and the increase of pH levels led to increases of both inorganic N and N-NO_3contents. Increases in pH levels caused N-NO_3levels to increase in both soils, however this occurrence happened because it increased the amount of mineralized N in the soil, seen that in all pH ranges in both soils practically all mineral N was in the form of N-NO_3.

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Introduction

Nitrification is performed by a specific group of nitrifying chemolithotrophic bacteria and archaea, where ammonium ions (N-NH_4) are oxidized to nitrate (N-NO_3), and occurs in several aquatic and terrestrial ecosystems (De Boer and Kowalchuk 2001; Head et al. 1993). In the soil, this is an important process that is directly involved in the availability of nitrogen (N) to plants, since plants will preferentially absorb N-NH_4 and N-NO_3. Despite being a readily available form to plants, N-NO_3 also carries a potential contamination risk, since it is not adsorbed by the negative charges of the solid fraction of the soil, thus being easily lixiviated to groundwater and surface water bodies contributing to eutrophication (Cherobaeva et al. 2011).

Nitrification is also responsible for soil acidification, and for this reason, together with the potential risk of contamination of N-NO_3, specific measures that reduce nitrification rates may contribute to a better quality of the soil. Soil pH is one of the factors that greatly impacts the N cycle, including nitrification and mineralization (Curtin and Trolove 2013; Sahrawat 2008), and it is believed that Nitrification has an optimal point at pH 8.0 and does not occur at a pH lower than 5.5 (Sahrawat 2008); however, it has already been proven that in extremely acidic soils nitrification may also occur (De Boer and Kowalchuk 2001; Gubry-Rangin, Nicol, and Prosser 2010; Head et al. 1993). Another factor

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that may interfere with nitrification refers to the species that composes the nitrifying community of the soil (Webster et al. 2005). Such interference occurs because nitrification is performed by a select group and these species possess great differences in both efficiency and adaptability to environmental conditions (Nicol et al. 2008; Yao, Campbell, and Qiao 2011).

In this sense, soils from different regions and origins may have distinct communities of microorganisms (Cherobaeva et al. 2011), thus being able to respond differently to environmental shifts. Yao, Campbell, and Qiao (2011) observed that by changing the soil reaction from alkaline to acid, the composition of nitrifying bacteria that dominated the process drastically changed.

Knowing that soils differ as to the composition of its nitrifying communities and each one of them respond differently to pH changes, the hypothesis of this study was that increases in soil's pH might increase nitrification rates, and that such increase would be lower in the originally acid soil (dystrophic). Thus, the aim of this study was to evaluate N mineralization of two red latosols, one eutrophic and other dystrophic, at different pH levels.

Materials and methods

Two soil samples (depth 0–20 cm) were collected in Jaboticabal/SP, Brazil (LV1) and Frutal/MG, Brazil (LV2), classified as eutrophic red latosol or Eutrudox, and dystrophic red latosol or Hapludox, respectively (Embrapa 2013; Soil Survey Staff 2014). Chemical characteristics (Raij et al. 2001) and texture (Camargo et al. 2009) of both samples are described in Table 1.

The experimental design was completely randomized in a factorial scheme 2 × 6 with three replicates, in which the first factor was both soils and the second factor referred to six pH levels determined with $CaCl_2$ (4.0, 4.5, 5.0, 5.5, 6.0, and 6.5), resulting in 36 plots. In order to reach these pH values, different doses of $Ca(OH)_2$ were added to the soils, which were sufficient to correct its acidity and increase the pH to the desired level. To this end, the acid neutralization curves for each soil were determined by the addition of 7 $Ca(OH)_2$ doses, with three replicates each. The applied doses of $Ca(OH)_2$ to achieve each pH level mentioned above are shown in Table 2.

A regression analysis was performed between doses and observed pH values, and based on the obtained regressions it was possible to calculate the amount of $Ca(OH)_2$ needed for each treatment. Soil characterization was not performed after the addition of $Ca(OH)_2$ doses in the treatments. N-NH_4 and N-NO_3 contents were measured at different days for the adjustment of the mineralization and nitrification models, in function of time, with the evaluated times being 0, 7, 14, 28, 42, 56 and 70 days of incubation.

The plots were made by weighting 50 g of each soil blended with the pre-calculated quantities of $Ca(OH)_2$. Samples were homogenized, placed in plastic pots (50 ml), moistened up to 60% of the water holding capacity and incubated under laboratory conditions at room temperature. Throughout the incubation period, the samples were daily moistened in order to keep soil humidity at 60%.

Mineral N (N_{min}) was determined according to the methods described in Cantarella and Trivelin (2001). The N_{min} was extracted with a KCl solution (1 mol L^{-1}) and in the obtained extract, we assessed ammonium (N-NH_4) and nitrate (N-NO_3) concentrations by means of the steam distillation

Table 1. Chemical attributes and texture of the soils used in the nitrogen mineralization experiment at different pH levels.

	g dm^{-3}	mg dm^{-3}			mmol$_c$ dm^{-3}							%	g kg^{-1}	
Soil[a]	SOM[b]	Resin-P	S SO_4^{2-}	pH $CaCl_2$	K^+	Ca^{2+}	Mg^{2+}	H+ Al	Al^{3+}	SB	CEC	BS	Clay	Sand
LV1	28	10	16	3,9	0,9	8	2	72	13	11	83	13	590	400
LV2	17	2	9	4,0	0,6	2	1	47	11	4	51	7	190	730

[a]LV1: eutrophic red latosol collected in Jaboticabal, São Paulo, Brazil; LV2: dystrophic red latosol collected in Frutal, Minas Gerais, Brazil.
[b]SOM: soil organic matter; resin P: soil available P extracted with anion exchange resin; S-SO_4^{2-}: soil available sulfate; K^+, Ca^{2+}, Mg^{2+}, Al^{3+}: exchangeable K, Ca, Mg and Al, respectively; H+ Al: total acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; Clay and Sand: levels of total clay and sand content, respectively.

Table 2. Mean values of pH $CaCl_2$ of the samples treated with $Ca(OH)_2$, for the construction of a neutralization curve, and the adjusted curves for each of the latosols used in this experiment.

Soil	$Ca(OH)_2$ Dose g 10 cm^{-3}	pH $CaCl_2$	Equation	F	R^2
LV1	0	3.89	Y = 90.079x + 3.9869	22.51**	0.9907
	0.0057	4.45			
	0.0113	5.10			
	0.0170	5.58			
	0.0226	6.13			
	0.0283	6.59			
	0.0339	6.87			
LV2	0	4.15	Y = 123.09x + 4.1903	9.48**	0.9917
	0.0037	4.55			
	0.0075	5.17			
	0.0112	5.66			
	0.0149	6.14			
	0.0186	6.47			
	0.0224	6.83			

[a]LV1: eutrophic red latosol; LV2: dystrophic red latosol.

method. The total mineral N (Ni) was obtained by the sum of N-NH_4 and N-NO_3 of the soil. The results of mineralized N (Ni at evaluated times – Ni at time zero) were adjusted to the exponential kinetic model of first order proposed by Stanford and Smith (1972):

$$N_m = N_0 \times \left(1 - e^{-kt}\right), \text{ in which:}$$

N_m: mineralized N; N_0: potentially mineralized N; k: constant of mineralization; t: incubation time.

The results regarding NO_3^- contents in the soil in function of incubation time were adjusted to the logistic growth model, based on the following formula.

$$NO_3 = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + e^{-k(t - t_0)}}, \text{ in which:}$$

NO_3: nitrate content of the soil; Y_{max}: maximum content of nitrate in the soil, upper asymptote; Y_{min}: minimum nitrate content, lower asymptote; t: incubation time; t_0: time corresponding to the highest nitrification rate in the soil, inflexion point; k: maximum intrinsic growth rate.

The levels of NO_3^- at 70 days of incubation were also adjusted to the logistic growth model of the general formula described above. However, the variation is given in function of soil pH levels and not in function of time, with the replacement of the variables t and t_0 by pH and pH_0. All models were chosen based on the behavior of the data and on its accuracy. The nonlinear regression analyses were performed using the Microsoft Excel 2016® software and the GRG2 method described by Lasdon et al. (1978)

Both NO_3^- and N_i contents at 70 days of incubation were submitted to a variance analysis and means were compared by the Tukey *post-hoc* test considering a 95% confidence interval. The models' adjustments were also evaluated by a variance analysis, by the determination coefficient (r^2) and by the mean absolute percentage error (MAPE).

Results

Both levels of mineralized N-NO_3 and total Ni up to day 70 of incubation were higher in LV1, and in both soils, the contents increased with higher pH levels (Table 3). In order to adjust the mineralized N during incubation, the difference between observed Ni contents and the initial Ni content in the soil was used, according to the methodology proposed by Stanford and Smith (1972). In the data adjustment of N-NO_3, only the levels of N-NO_3 in the soils were used, including the ones observed in time zero.

Table 3. Nitrate and total inorganic nitrogen concentrations of two soils after 70 days of incubation in a laboratory setting.

Treatments[a]		$N\text{-}NO_3^{-b}$	Ni
Soil	pH	(mg kg^{-1})	(day^{-1})
LV1	4.0	59.5 e[c]	62.5 e
	4.5	60.5 de	62.8 e
	5.0	62.3 d	66.9 d
	5.5	67.3 c	73.2 c
	6.0	79.7 b	84.5 b
	6.5	84.4 a	87.9 a
Mean		68.9 A	73.0 A
LV2	4.0	41.0 e	43.6 e
	4.5	44.5 de	47.1 e
	5.0	45.9 d	50.1 d
	5.5	49.9 c	53.5 c
	6.0	61.7 b	63.7 b
	6.5	71.7 a	73.7 a
Mean		52.5 B	55.3 B

[a]LV1: eutrophic red latosol; LV2: dystrophic red latosol; pH: value of soil pH measured in $CaCl_2$, obtained after the correction with $Ca(OH)_2$.
[b]$N\text{-}NO_3^-$: soil nitrate content; Ni: soil mineral nitrogen content (sum of $N\text{-}NH_4^+$+$N\text{-}NO_3^-$ levels).
[c]Means followed by different letters indicate significant differences by the Tukey's test ($p < 0.05$). Lowercase letters were used in the comparison between pH effects in the soil, while uppercase letters were used in the comparison of soil types.

Table 4. Parameters of the kinetic equation of first order of Stanford and Smith obtained by the adjustment of mineralized nitrogen data in function of incubation time, in an experiment with two soils and six pH conditions.

Treatments[a]		N_0[b]	k	T ½		
Soil	pH	(mg kg^{-1})	(day^{-1})	(days)	R^2	dw
LV1	4.0	39.8	0.024	29.2	0.93	0.99
	4.5	40.6	0.024	29.1	0.78	0.96
	5.0	36.6	0.055	12.7	0.81	0.96
	5.5	40.8	0.044	15.8	0.82	0.96
	6.0	52.0	0.050	13.9	0.81	0.96
	6.5	61.3	0.032	21.6	0.88	0.98
LV2	4.0	38.2	0.020	34.8	0.98	0.99
	4.5	73.2	0.008	86.6	0.99	0.99
	5.0	43.9	0.018	38.6	0.91	0.98
	5.5	35.7	0.044	15.7	0.87	0.98
	6.0	41.3	0.056	12.3	0.89	0.98
	6.5	60.6	0.040	17.3	0.98	0.99

[a]LV1: eutrophic red latosol; LV2: dystrophic red latosol; pH: value of soil pH measured in $CaCl_2$, relation soil: solution 1:2.5, obtained after the correction with $Ca(OH)_2$.
[b]N_0: potentially mineralizable nitrogen; k: mineralization constant; T ½: half-life time; R^2: determination coefficient; dw: Willmot's concordance index.

In LV1, the values of potentially mineralizable N (N_0) increased along with pH, especially from pH 5.5, which was not observed in LV2, where the highest value of N_0 was observed in pH 4.5, together with the lowest value of k (Table 4). Lower values of k and higher values of T ½ indicate that the mineralization rate of the soils with lower pH levels was slower (Table 4). Generally, the observed values of k in treatments with inferior values of pH were lower than in the other treatments.

The logistic growth model was chosen because it presented greater similarity with the behavior of $N\text{-}NO_3$ levels in function of time, besides having higher accuracy measured by the MAPE in

Table 5. Parameters of the logistic growth model obtained in the adjustments of the nitrate content in the soil in function of incubation time, in an experiment with two types of soils and six conditions of pH.

Treatments[a]		Ymin[b]	Ymax	t_0			MAPE
Soil	pH	(mg kg^{-1})	(mg kg^{-1})	(days)	k	R^2	%
LV1	4.0	0.0	57.6	8.7	0.073	0.98	3.8
	4.5	0.0	56.1	9.1	0.104	0.94	9.2
	5.0	0.0	56.2	7.2	0.147	0.92	9.5
	5.5	0.0	58.5	7.5	0.130	0.88	11.0
	6.0	0.0	74.5	12.6	0.078	0.96	7.4
	6.5	0.0	80.4	16.1	0.071	0.94	11.4
LV2	4.0	0.0	39.5	16.6	0.075	0.98	5.6
	4.5	0.0	43.5	19.1	0.071	0.98	8.1
	5.0	0.0	40.8	13.5	0.100	0.95	6.8
	5.5	7.7	44.7	18.2	0.197	0.97	7.3
	6.0	0.0	53.9	16.8	0.111	0.95	10.1
	6.5	0.0	67.0	20.0	0.149	0.98	22.0

[a]LV1: eutrophic red latosol; LV2: dystrophic red latosol; pH: value of soil pH measured in $CaCl_2$, obtained after the correction with $Ca(OH)_2$.
[b]Ymin: estimated minimum value of nitrate in the soil; Ymax: estimated maximum value of nitrate in the soil; t_0: time corresponding to the highest nitrification rate; k: maximum rate of nitrification; R^2: coefficient of determination; MAPE: mean absolute percentage error.

Table 6. Parameters of the logistic growth model obtained in the adjustments of the nitrate content in the soil at 70 days of incubation in function of pH values in an experiment with two soils and six conditions of pH.

	Ymin[b]	Ymax				MAPE
	(mg kg^{-1})	(mg kg^{-1})	pH_0	k	R^2	%
LV1	60.1	85.6	5.7	4.03	0.99	0.99
LV2	42.0	82.2	6.0	2.31	0.99	0.99

[a]LV1: eutrophic red latosol; LV2: dystrophic red latosol.
[b]Ymin: estimated minimum value of nitrate in the soil; Ymax: estimated maximum value of nitrate in the soil; pH_0 : value of pH in $CaCl_2$ where the curve's inflexion occurs; k: maximum intrinsic growth rate; R^2: coefficient of determination; MAPE: mean absolute percentage error.

comparison to linear models. It was possible to observe an increase of Ymax in both soils, due to the elevation of soil pH (Table 5). The values of t_0 indicate the moment when half of the N-NO_3 accumulation occurred (Ymax – Ymin), while k indicates the speed of such buildup, considering that lower values indicate gradual variation and higher values indicate abrupt accumulation. Thus, in treatments with pH 6.0 and 6.5 in LV1, a greater and gradual accumulation was verified throughout incubation, seen that t_0 values were high and k values were low (Table 5). In LV2, the values of t_0 and k oscillated without a defined trend in different pH values.

In the adjustment of the accumulated N-NO_3 throughout incubation in function of pH values, the model parameters are from the same model used in the N-NO_3 adjustment in function of time, with the replacement of time by the soil's pH value. Therefore, increases in soil's pH had a greater effect in LV2, where it led to an increase of 40 mg kg^{-1} (difference between Ymax and Ymin) in comparison to 25.5 mg kg^{-1} in LV1 (Table 5). In LV1, the variation in NO_3 contents in function of pH was smaller and more abrupt, while in LV2 the variation is larger and more smooth (Table 6, Figure 1).

Discussion

The highest amounts of mineralized Ni and N-NO_3 at 70 days of incubation observed in LV1 occurred mainly due to the greater SOM content in this soil (Table 1), for which the mineralization is the main source of Ni (Gava et al. 2006). Increased levels of Ni and N-NO_3 in function of pH levels were more pronounced from pH 5.5 and occurred due to a limitation that acid soils impose on soil microorganisms (Rousk, Brookes, and Baath 2009). Although it is believed that both acidity and low

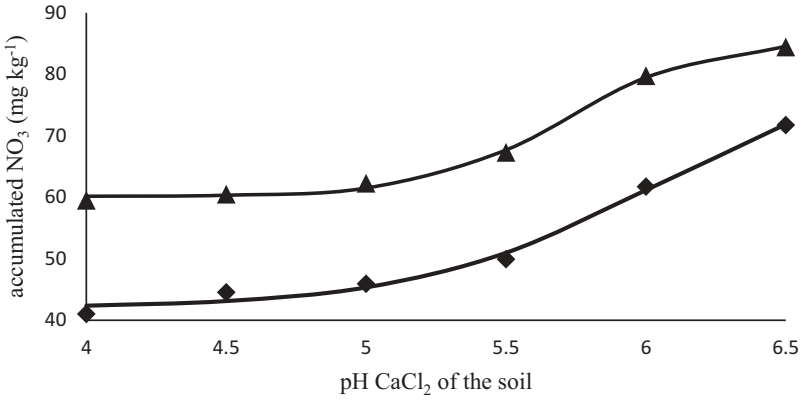

Figure 1. Observed data and adjusted logistic model (lines) of the nitrate levels at 70 days of incubation in function of soil's pH, in samples of eutrophic red latosol (▲) and dystrophic red latosol (♦).

pH levels in soils are limiting factors to nitrification (Sahrawat 2008), such limitation did not prevent that at 70 days of incubation, and almost all Ni was found as N-NO_3 (Table 3). We hypothesized that this fact was attributed to the adaptation of the nitrifying communities to acid soils, as observed by Parkin, Sexstone, and Tiedje (1985) and Yao, Campbell, and Qiao (2011). In addition, slight changes of nitrification rates may have occurred due to increased microbial activity in the soils containing more calcium, even though the amounts added were not very high (Table 2).

Soil microbial communities respond to increases of several nutrients in the soil solid/liquid phases. The addition of Ca in the form $Ca(OH)_2$ – performed to achieve the desired pH levels in this study, may have been one of the factors affecting the increased nitrification rates, observed in both soils. As more Ca occupies the negative charges in the soil's solid phase, not only the pH is elevated, but also the microorganisms are stimulated due to higher availability of Ca (Macura and Stotzky 1980), seen that this micronutrient is highly demanded by microorganisms (Groffman and Fisk 2011). Therefore, with increased microbial activity, higher nitrification rates were observed. However, as pH increases are known to affect nitrification rates (Ramos et al. 2017; Sahrawat 2008), it is particularly difficult to differentiate the effects of pH and Ca in the N mineralization rates throughout the experiment.

In LV1, increased values of N_0 from pH 5.5 occurred according to Ni contents at 70 days of incubation (Tables 3 and 4) and was given by the limitation that severe acidity causes in the microorganisms metabolism (Rousk, Brookes, and Baath 2009). This is a limitation found in low values of k when the pH was 4.0 and 4.5 (Table 4). This effect of the pH was not observed in LV2, due to difficulties found in the model adjustment, verified by high values of T ½ (Table 4), thus indicating that incubation time was not enough for the definition of N_0 (Stanford and Smith 1972).

Nitrification had a different behavior from mineralization, with a slow initial period; therefore, data were adjusted to the logistic model. In both soils, Ymax increased along with the pH, due to the strong dependency of the nitrification process with soil's pH (Sahrawat 2008). Another factor that contributed to such increases was the effect of pH in N mineralization (Table 3), seen that mineralization leads to the formations of N-NH_4, which is the substrate of nitrification. The lowest observed values of t_0 in LV1 (Table 5) coincide with the highest values of k of the Stanford Smith adjustment for this soil in most of the treatments (Table 4), indicating that both mineralization and nitrification occur more rapidly in this soil. Despite the fact that the nitrifying communities converted almost all Ni in N-NO_3 by the end of the incubation, at the beginning of this period nitrification occurred slowly, especially in the treatment with pH 6.5, due to the high value of t_0, which is attributed to the strong change in pH in relation to the original condition (Table 1).

When evaluating the effect of pH variation in $N\text{-}NO_3$ contents (Table 6), it was possible to observe that in both soils, the maximum values of $N\text{-}NO_3$ (Ymax) were similar, probably due to the availability of existing mineral N, which may act as a limiting factor for mineralization (Sahrawat 2008). The lowest value of Ymin observed in LV2 may be a result of smaller amounts of SOM, which reduced the quantity of mineralized N (Braos et al., 2016) and also by the lower efficiency of the microorganisms involved in the process (Nicol et al. 2008). Values of Ymin close to 60 and 40 contradict the studies that suggested that nitrification occurs only above pH 5.0 (Sahrawat 2008). The values of pH_0 were similar, but the value of k was higher in LV1 (Table 6), which indicates that an abrupt variation of $N\text{-}NO_3$ levels, while in LV2 the variation was smoother (Figure 1). In this sense, despite the values of Ymax having been shown to be similar, in LV2 this value is reached in increased pH levels in comparison to LV1. This result is an effect of the better adaptation of the nitrifying communities of LV1 to the range of pH levels, as suggested in several studies (Jiang et al. 2015; Parkin, Sexstone, and Tiedje 1985; Yao, Campbell, and Qiao 2011).

Conclusion

In both soils, pH influenced nitrification rates at the beginning of the incubation period. However, as the nitrifying communities have apparently adapted to the new conditions of pH, by the end of the experiment all mineral N was found as $N\text{-}NO_3$. Thus, the main factor that acted as a regulator of the amount of $N\text{-}NO_3$formed at the final stage of incubation was the N mineralization itself, i.e., the passage from organic N to $N\text{-}NH_4$. In the dystrophic red latosol, the slow period of nitrification at the beginning of incubation was higher, but despite the stage of adaptation was longer in this soil, the microorganism communities were able to adapt to new pH condition and nitrify all of the available existing N.

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Conflict of Interest

The authors declare no conflicts of interest.

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