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Research Article

Conversion of ammonium to nitrate and abundance of ammonium-oxidizing-microorganism in Tropical soils with nitrification inhibitor

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ABSTRACT: The use of nitrification inhibitors (NIs; dicyandiamide - DCD) is an alternative to reduce oxidation of ammonium (NH_4^+-N) to nitrate (NO_3^--N) in the soil, reducing NO_3^--N losses from fertilization practices. Based on the hypothesis that DCD reduces conversion of NH_4^+-N to NO_3^--N in tropical soils and inhibits ammonia oxidizing microorganisms (AOM) abundance, soils from the Piracicaba region, São Paulo, with different textures (sand, loam and clay) were incubated with ammonium sulphate (AS) and DCD. Contents of NH_4^+-N, NO_3^--N, soil pH, and AOM abundance were quantified periodically. Ammonium sulphate increased AOM abundance in all soils, but AS+DCD presented AOM abundances similar to the control. During 90 days of incubation, the effectiveness of DCD in reducing NO_3^--N production was 1.8, 86.4, and 145.6 mg kg^{-1}, while the effectiveness of DCD in reducing AOM abundance was 1.2, 3.0 and 2.3 × 10^{-3} g soil^{-1} for sandy, loamy, and clayey soils, respectively. DCD effectiveness was greater in loamy and clayey soils due to the naturally low nitrification in sandy soils. Application of AS treated with DCD showed potential not only to reduce NO_3^--N production in loamy and clayey soils, but also to decrease the soil nitrification rate. Overall, DCD was effective in reducing AOM abundance and conversion of NH_4^+-N to NO_3^--N in loamy and clay soils evaluated here. The increase in clay content directly influences DCD effectiveness in reducing conversion of NH_4^+-N to NO_3^--N.

Keywords: DCD, nitrogen losses, ammonium sulphate, fertilizer

Introduction

Nitrogen (N) is a plant nutrient that plays a key role in sustainability and agricultural production (Yan et al., 2014). In recent years, N losses from fertilization practices have become an issue due to increased leaching of nitrate (NO_3^--N), emission of nitrous oxide (N_2O) and release of (H^+) proton, contributing to global warming, biodiversity loss, soil acidification and water eutrophication (Chen et al., 2015). Losses of N from fertilizer under tropical conditions range from 20 to 30 % (Cantarella et al., 2008), depending of N sources, soil moisture and soil pH (Gallucci et al., 2018).

Nitrification is the conversion process of ammonium (NH_4^+-N) to NO_3^--N, separated in two steps: (i) first is ammonia oxidation to nitrite, carried out by groups of microorganisms known as ammonia-oxidizers; (ii) second is oxidation of nitrite (NO_2^--N) to NO_3^--N, carried out by groups of nitrite-oxidizing bacteria (Bernhard, 2010). The use of nitrification inhibitors (NIs) is an alternative to reduce oxidation of NH_4^+-N to NO_3^--N in the soil.

Dicyandiamide (DCD, $C_2H_4N_4$) is a NI that limits or reduces NO_3^--N formation from NH_4^+-N or ammonium-producing fertilizers (Rodgers, 1986). Previous studies indicate that DCD regulates soil N transformations and increases plant productivity (Yang et al., 2016). However, the soil texture effect on DCD effectiveness is poorly understood, especially under tropical soil conditions, which present a peculiarity to the predominance of low activity clay and low soil organic matter (SOM) content (Camargo et al., 2013), which might interfere on DCD performance.

DCD is capable of affecting the active site of ammonia oxidizing microorganisms (AOM), responsible for the nitrification process or conversion of NH_4^+-N to hydroxylamine and further to NO_3^--N (Di et al., 2009; Gong et al., 2013). Recently, two groups of different microorganisms (ammonia-oxidizing bacteria – AOB and ammonia oxidizing archaea – AOA) have been studied predominantly in the soil as major responsible for autotrophic ammonia oxidation in terrestrial ecosystems (Leininger et al., 2006). We carried out an incubation study with application of DCD and ammonium sulphate in tropical soils with different textures from the Piracicaba region, São Paulo, to test the hypothesis that DCD inhibits AOM abundance and reduces conversion of NH_4^+-N to NO_3^--N.

Materials and Methods

The experiment was carried out under laboratory conditions with the temperature controlled at 25 ± 1 °C, using a factorial design 3 × 3, represented by three soil textures (sand, loam and clay) and three N fertilizations (1: Control: No-N; 2: ammonium sulphate-AS; and 3: AS + DCD), with three replications.

Soil samples were collected from the 0.0 - 0.2 m surface layer of sugarcane areas in the Piracicaba region, São Paulo (latitude 22°42′30″ S; longitude 47°38′00″ W; altitude 546 m). The samples were air-dried, sieved through a 2 mm mesh and analyzed for soil characterization. Sandy, loamy and clayey soil were classified as an Entisol, Cambisol and Ferralsols according to FAO soil classification (FAO, 2015), and chemical and physical characterized according to Van Raij et al. (2001) and Camargo et al. (2009), Table 1.

Dry soil samples (50 g) were transferred to plastic bottles (500 mL capacity), moistened to 70 % of water holding capacity (WHC) and kept in the dark at 25 °C during ten days to activate microbial activity. After the pre-incubation period, a solution containing AS was homogeneously distributed and incorporated (solid; rate 300 mg N kg^{-1}). When present, DCD was applied at a rate corresponding to 15 % of N (in that case, the N amount in the AS was equilibrated to 85 % of total). The bottles were sealed with a plastic film to prevent water loss, but allow gas exchange and placed in an open laboratory environment for 98 days. Samples were weighed weekly and moistened when necessary to keep the water content at approximately 70 % of WHC.

Soil samples were collected after the 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th}, 42^{th}, 63^{th} and 98^{th} day of incubation (DOI). Deionized water (125 mL) was added to the bottles (soil: solution ratio of 1:2.5) to measure soil pH, following measurements in triplicate. Inorganic N was extracted using an additional solution of KCl (125 mL; 4 mol L^{-1}), which was added in bottles (for a 1:5 soil solution of 2 mol L^{-1} KCl), followed by shaking in an orbital shaker for 1 h and filtration through filter paper N° 42. NH_4^+-N and NO_3^--N were determined sequentially in the extract by steam distillation following the procedures described in Cantarella and Trivelin (2001).

AOM abundance in the soil was determined as most probable number (MPN) (Cochran, 1950), after the 1^{st}, 21^{st}, 42^{th}, 63^{th} and 98^{th} DOI, using 10 g of soil and following a dilution in sodium chloride solutions (NaCl; 1 %), according to Sarathchandra (1978). AOM abundance was determined in the same solution as mineral N. The MPN is considered a consolidate methodology to estimate microorganism abundance in the soil and is widely used (Nakatani et al., 2011; Ruppel and Makswitat, 1999).

DCD effectiveness was expressed as inhibitor efficiency (IE) and calculated as a mean difference of NO_3^--N content and AOM abundance between AS and AS + DCD for each period of incubation.

Descriptive statistics were assessed and assumptions of normality and homogeneity of variance were evaluated according to Shapiro-Wilk and Bartlett-test, respectively. Soil textures and N fertilizations were assessed using the analysis of variance based on the F-Test statistic, when the F-test was significant ($p < 0.05$), the soil texture and N fertilizations were compared using the Tukey test ($p < 0.05$).

Table 1 – Chemical and physical properties of sandy, loamy and clayey soils.

Soil texture	Soil classification	pH	CEC	TN	OC	Sand	Silt	Clay
		$CaCl_2$	$mmol_c$ dm^{-3}	g kg^{-1}				
Sandy	Entisol	5.2	44.3	0.5	18.1	927	2	71
Loamy	Cambisol	5.9	100.7	1.5	35.2	290	480	230
Clayey	Ferralsols	5.9	158.1	1.2	60.3	206	94	700

CEC = cation exchange capacity; TN = Total nitrogen; OC = Organic carbon. Soil classified according to FAO classification (FAO, 2015).

Results

The content of NH_4^+-N and NO_3^--N remained stable over the incubation period in the sandy soil for all treatments, demonstrating limited oxidation of NH_4^+-N to NO_3^--N (Figures 1A and 1D). On the other hand, there was a rapid conversion of NH_4^+-N to NO_3^--N in loamy (Figures 1B and 1E) and clayey soils for AS (Figures 1C and 1F). Opposite to the NH_4^+-N content, which reduced over the incubation period in the loamy and clayey soils, the content of NO_3^--N increased over the incubation period in both soils, represented by a negative correlation of -0.94 and -0.82 ($p < 0.05$) between NH_4^+-N and NO_3^--N, respectively. The increase in NO_3^--N content over the incubation period followed the order clayey > loamy > sandy soil (Figures 1D, 1E and 1F).

The difference between NO_3^--N content in AS and AS + DCD can be associated with the use of DCD. This approach allowed to calculate the amount of NO_3^--N that was not produced because of the inhibition by DCD. The IE in NO_3^--N was 1.8, 86.4, and 145.6 mg kg^{-1} in sandy, loamy and clayey soil, respectively (Figures 1D, 1E and 1F).

Besides inhibiting the nitrification process, treating AS with DCD also showed potential in reducing the proton (H^+) release, especially in the loamy and clayey soils (Figures 1H and 1I). In the sandy soil, there was little variation in soil pH over incubation, following the limited conversion of NH_4^+-N to NO_3^--N, confirmed by the absence of correlation between soil pH and NO_3^--N production ($r = 0.11$; $p > 0.05$) (Figure 1G). In the loamy soil, soil pH remained stable in the control and AS + DCD, but reduced significantly in AS (Figure 1H). In the clayey soil, which presented the highest nitrification rate, soil pH followed the sequence AS + DCD > AS (Figure 1I).

AS application increased AOM abundance in the first two weeks in the sandy soil, and during the whole incubation period in the loamy and clayey soils (Figures 1J, 1K and 1L). The application of AS with DCD reduced AOM abundance to levels similar to the control with no difference in the sandy and loamy soils during 98 DOI ($p > 0.05$; Figures 1J and 1K).

In the clayey soil, AOM abundance was similar between control and AS + DCD for 21 DOI, but then AS + DCD increased the AOM abundance when compared to the control (Figure 1L). The IE of DCD in AOM abundance was 1.2, 3.0 and 2.3 mg kg^{-1} in sandy, loamy and clayey soils, respectively (Figures 1J, 1K and 1L).

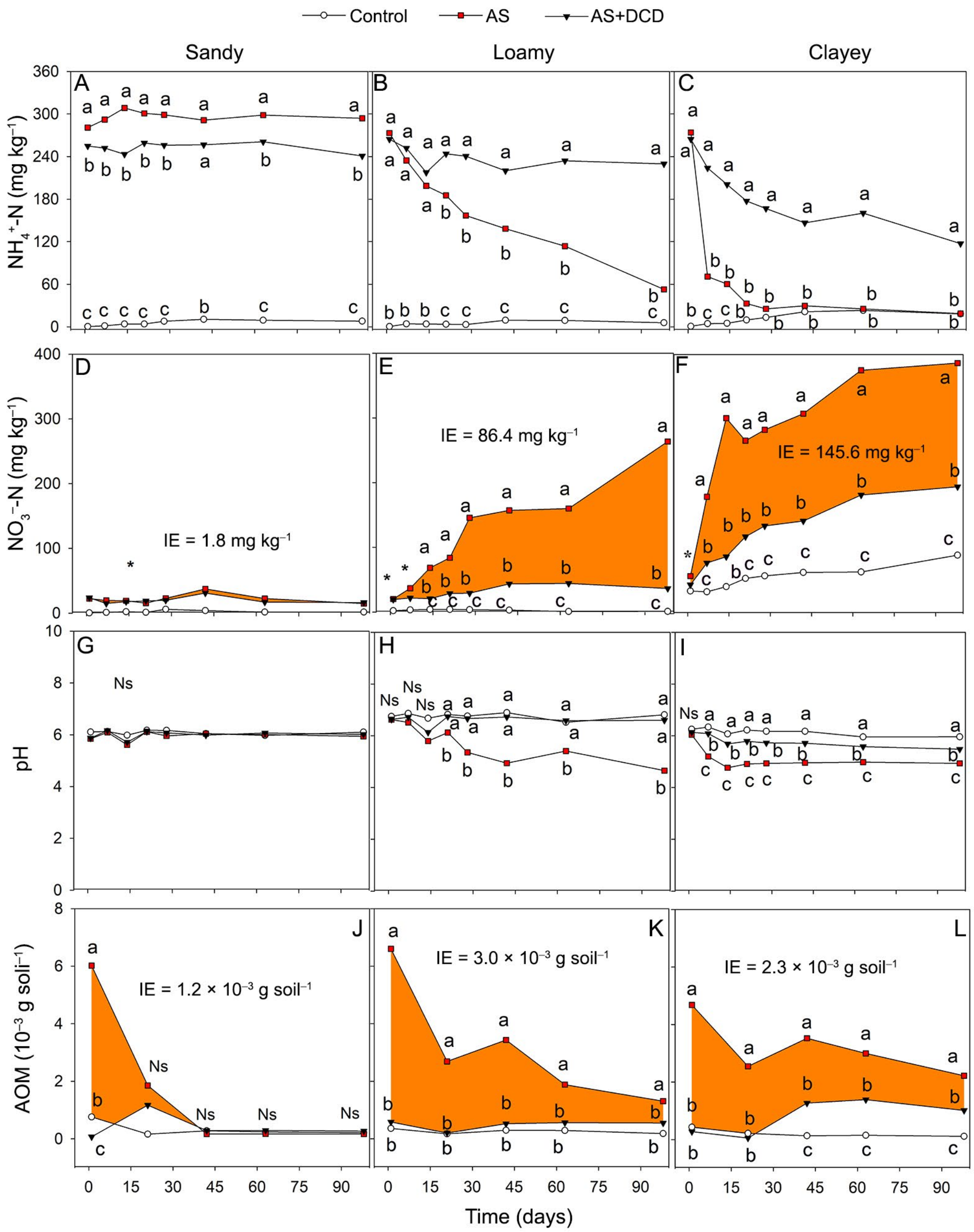

Figure 1 – Daily ammonium (NH_4^+-N; mg kg^{-1}), nitrate (NO_3^--N; mg kg^{-1}), pH and ammonia oxidizing microorganisms (AOM; 10^3 g soil^{-1}) in sandy (A, D, G and J), loamy (B, E, H and K) and clayey soil (C, F, I and L) with ammonium sulphate (AS), isolated and associated with dicyandiamide (DCD). Treatments were compared by the Tukey test and results represented by lowercase letters ($p < 0.05$); Ns = Not significant; *Significant difference between control and AS/AS+DCD; IE = Inhibitor efficiency, represented by orange color in NO_3^--N and AOM Graph.

Discussion

In our study, nitrification was lower in the sandy soil than in loamy and clayey soils, possibly due to the low clay and organic matter (OM) content in the sandy soil. The low OM content in the sandy soil may have limited the conversion of NH_4^+-N to NO_3^--N by AOM. The limited nitrification observed in the sandy soil is confirmed by little variation in soil pH during incubation, since nitrification releases H^+ to the soil solution in the first step of the nitrification process (Bernhard, 2010). The limited nitrification in the sandy soil seems to be the reason for the low DCD effectiveness, which reduced 1.8 mg kg^{-1} the production of NO_3^--N during the incubation period and decreased only 1.2 × 10^{-3} g soil^{-1} of AOM abundance.

The greater DCD effectiveness in loamy and clayey soils are associated with higher nitrification of both soils. However, DCD effectiveness decreases over time due to DCD degradation that usually occurs in the soil (Barth et al., 2001; Clay et al., 1990), and DCD consumption by the soil biota (Hauser and Haselwandter, 1990). The positive effect of DCD to reduce nitrification in loamy and clayey soils also kept the soil pH closer to control. This is an important benefit of nitrification inhibitors associated with N fertilizers, since soil acidification by continuous application of N fertilizer is a concern worldwide (Schroder et al., 2011). Although it was not evaluated in our study, the use of nitrification inhibitors has the potential to reduce nitrate leaching (Di et al., 2003) and N_2O emission (Dai et al., 2013; Shamsuzzaman et al., 2016); nevertheless, it can increase NH_3 emission when mixed to urea-based fertilizers (Soares et al., 2016). In addition, DCD applied to fertilizer can play a role as a sink for methane in tropical humid climate (Jumadi et al., 2008). The N saved due to the use of nitrification inhibitors can be absorbed by plants, improving dry matter and N use efficiency (Zaman and Blennerhassett, 2010). In fact, several studies have shown the potential of nitrification inhibitors to increase yields of major crops (Yang et al., 2016; Li et al., 2018).

The use of DCD reduced AOM abundance in all soil textures, demonstrating DCD effectiveness in reducing AOM abundance in tropical soils evaluated here. Previous studies reported the potential of DCD to reduce AOM when applied with ammonium fertilizers (Yan et al., 2014). AOM, represented by AOB and AOA, is responsible for autotrophic ammonia oxidation (O'Callaghan et al., 2010); however, AOA seems to play a more important role in the nitrification process than AOB in acidic soils, such as those of the tropical regions (Zhang et al., 2012). More importantly, AOA is more sensitive to DCD addition (Di et al., 2009). The low amount of water available in the sandy soil might also be altered AOM abundance in our study, as described by Stark and Firestone (1995). Sandy soils present much lower water amounts than clayey soils do (Andrade and Stone, 2011), which affects the population of microorganisms. The results reported here support the hypothesis that DCD inhibits AOM abundance in tropical soils, similar to previous evidence obtained under temperate conditions.

Conclusion

DCD is effective in reducing AOM abundance and conversion of ammonium to nitrate in loamy and clayey soils from the tropical region of Piracicaba, São Paulo. The increase in clay content directly influences DCD effectiveness to reduce conversion of ammonium to nitrate.

The DCD effectiveness is reduced in sandy soils that naturally present limited nitrification. The use of DCD also showed potential in reducing soil acidification by N fertilization, which seems to be important in tropical soils that present significant soil acidity-related problems (Lopes and Guimarães, 2016).

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Authors' Contributions

Conceptualization: Barth, G.; Cardoso, E.J.B.; Vitti, G.C. Data acquisition: Barth, G. Data analysis: Almeida, R.F.; Otto, R.; Barth, G. Writing and editing: Almeida, R.F.; Otto, R.; Barth, G.; Cardoso, E.J.B.

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