Deposition and Transformation of Nitrogen after Soil Fumigation with Ethanedinitrile

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Abstract. A novel methyl bromide alternative, ethanedinitrile (EDN), has been reported to be efficacious against soil-borne pathogens, weeds, and plant-parasitic nematodes. Degradation products of EDN include NH_4^+ and NH_3 , but it is currently unknown at what quantities these degradation products are being released into the soil at a given use rate of EDN. To address this issue, field studies were performed using the raised-bed plasticulture system. Deposition of NH_4^+ and NO_3^- in top 0–15-, 15–30-, and 30–45-cm soils were evaluated 3 weeks after fumigation with EDN applied at 336, 448, and 560 $\text{kg}\cdot\text{ha}^{-1}$. Change of pH and transformation of NH_4^+ to NO_3^- in top 0–15- and 15–30-cm soils were tracked weekly after fumigation with EDN at 448 $\text{kg}\cdot\text{ha}^{-1}$ for 10 weeks. This study found that fumigation with EDN significantly increased soil pH of the top 0–15-cm soil and soil NH_4^+ in top 0–15- and 15–30-cm soils, but soil NO_3^- was unaffected. Nitrification process in top 0–15-cm soil was inhibited by fumigation with EDN for at least 7 weeks. These results indicate that N deposited by fumigation with EDN could be an important preplant N source for crop production, and the inhibition of nitrification could help mitigate nitrate leaching. This study provides helpful information for quantification of N deposited from fumigation with EDN.

Due to the ban on methyl bromide (MBr), the efficacy of old and new soil fumigants have been examined and developed further. A novel soil fumigant not currently labeled in the United States, ethanedinitrile (EDN), was initially examined for use as a fumigant to control insect pests in stored grain (Hooper et al., 2003). EDN has since been examined as a soil fumigant in the United States as well as other countries with acceptable efficacy against weed pests, plant-parasitic nematodes, and soil-borne pathogens in a variety of crops such as tomato, carrot, and strawberry (Chung et al., 2007; Mattner et al., 2006; Park et al., 2014; Stevens et al., 2019). EDN has a high vapor pressure (515 kPa) and low boiling point (-21.1°C), which make it an ideal fumigant.

The chemical formula of EDN is C_2N_2 , and some degradation products are NH_4^+ , NH_3 , CO_2 , and HCO^- . It is currently un-

known at what quantities plant-available nitrogen species are being released into the soil at a given use rate of EDN. The deposition of ammonium by EDN could be beneficial, because ammonium is commonly used as a source of nitrogen in fertilizer blends applied by vegetable producers. However, toxicity to some vegetable crops can occur because of an overabundance of ammonium (Simonne et al., 1992). Furthermore, ammonium is converted to nitrate in the soil by nitrifying bacteria. Under conditions that predominate in Florida, most of the soil N is quickly converted to NO_3^- , regardless of the originally applied form (Zotarelli et al., 2009). As a negatively charged ion, nitrate may not be readily retained by soil particles (Scharf, 2015). Nitrate also moves freely with water, because it is highly soluble. These characteristics lead to increased tendency of nitrate leaching and runoff, and potential surface water and groundwater contamination (Marchi et al., 2016), which could harm livestock and lead to eutrophication in aquatic ecosystems (Rios et al., 2013).

In Florida, many high-value crops such as tomato, pepper, and watermelon require high inputs of fertilizer and irrigation on sandy soils that have low nutrient and water retention capacity. These crops are typically grown in fumigated soil, on plastic mulch;

and drip irrigation is used to deliver water and fertilizer, which increases efficacy of nutrient delivery and reduces evaporation (Liu et al., 2020). Because water delivers the fertilizer, it is increasingly important to keep water primarily in the root zone to reduce leaching and runoff, which could lead to higher production costs and negative environmental impacts. Florida vegetable producers are encouraged to enroll in the best management practices (BMPs) program, which is proven to be the most effective means for proper water and fertilizer use, and for maintaining or improving the quality of ground and surface waters (FDACS, 2015). For Florida vegetable producers to comply with BMPs and mitigate potential of nitrate leaching into the groundwater, it is important to assess the deposition of nitrogen by EDN.

One area of concern regarding soil fumigants is their impact on soil microbes, some of which are responsible for nitrogen transformation (Ibekwe et al., 2001). Generally, a significant increase of ammonium is observed immediately following application of a soil fumigant because of the mineralization of killed microbial biomass (De Neve et al., 2004; Shen et al., 1984). Nitrification is also impacted from the loss of nitrifying bacteria, resulting in reduced nitrate and a further accumulation of ammonium (Duniway, 2002; Jenkinson and Powlson 1970; Macnish 1986; Shen et al., 1984; Zhang et al., 2011). Yan et al. (2013) found that fumigation with MBr and methyl iodide inhibited nitrification by 43.4% and 37.2%, respectively, for up to 4 weeks. Another study found that fumigation with 1,3-dichloropropene inhibited nitrification for 8 weeks (Zhang et al., 2011). Preliminary data gathered during efficacy studies with EDN indicated that plant-available nitrogen species, primarily ammonium, were increasing after EDN application—but confounding factors did not allow for investigation of this subject during these experiments (Stevens et al., 2019). At this time, it is unclear what effect an ammonium-depositing soil fumigant will have on soil nitrogen transformations and total nitrogen deposition. This study aimed to assess ammonium deposition and its subsequent transformation to nitrate after application of EDN.

Materials and Methods

A field trial was conducted at the University of Florida North Florida Research and Education Center (NFREC) in Quincy, FL (lat. 30.54°N , long. 84.60°W) and was repeated three times: Spring 2018, Fall 2018, and Spring 2019. Duration, soil type and taxonomy, average soil temperature, and total rainfall of the three field seasons were listed in Table 1. Before the establishment of this study, each field was fallowed for at least one growing season and no fertilizer was applied. The experimental design was a randomized complete block design with four replications. Treatments included fumigation with EDN (99% liquid product, molecular weight 52.03 g/mol) (Lučební závody

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Table 1. Duration, soil type and taxonomy, average soil temperature (15 cm), and total rainfall of the three field seasons including Spring and Fall 2018 and Spring 2019 in Quincy, FL.

Season	Duration	Soil type	Soil taxonomy	Soil organic matter	Avg soil temp °C	Total rainfall mm
Spring 2018	28 Mar.–6 May	Orangeburg–Norfolk complex	fine-loamy, kaolinitic, thermic Typic Kandiodults	0.79%	21.8	326
Fall 2018	23 Aug.–1 Oct.	Dothan–Fuquay complex	loamy, kaolinitic, thermic Arenic Plinthic Kandiodults	0.67%	24.5	244
Spring 2019	11 Mar.–20 May	Orangeburg–Norfolk complex	fine-loamy, kaolinitic, thermic Typic Kandiodults	0.87%	20.0	224

Draslovka, Kolín, Czech Republic) at 336, 448, and 560 kg·ha⁻¹. The EDN molecule is roughly 53% nitrogen. All treatments were applied as a liquid, which quickly vaporizes, under 0.32-mm thick, white-on-black, totally impermeable film (Guardian Agricultural Plastics Corporation, Tampa, FL), using shank injection 25 cm below the soil surface with two “super sealer” shanks (Wiese Industries, Perry, IA) spaced 25 cm apart. Soil was cultivated to a depth of 25 cm before fumigation, and soil moisture was at least 75% field capacity. Fields used for these experiments were maintained in a clean, fallow state for at least 1 month before fumigation. The black-and-white side of the film showed for Spring and Fall seasons, respectively, which is typical of commercial production. Bed formation, fumigant application, and plastic deployment were accomplished with a single-row, combination bed press, where all three operations are performed at the same time. Bed dimensions of experimental plots were 76.2 cm wide and 20 cm high, with 1.8-m row spacing. No crops were planted into experimental plots, and no irrigation water was added after fumigation.

Two separate sampling regimes and analyses were performed on established experimental plots. One was established to investigate where and at what quantity plant-available nitrogen species may be deposited as impacted by EDN use rate. Another was established to determine how disruption of soil microbes by EDN application may alter the transformation of nitrogen species deposited by EDN.

For the nitrogen deposition study, two top 45-cm soil samples with a diameter of 4 cm were taken in each plot, in the center of each bed, with a Geoprobe (Geoprobe Systems, Salina, KS) 1 day before (“Pre”) and 3 weeks after (“Post”) fumigation. Each soil sample was separated into 0–15-cm, 15–30-cm, and 30–45-cm sections. The two samples from each plot were then thoroughly mixed as a composite sample. Soil pH, NO₃⁻, and NH₄⁺ at each depth were analyzed by a commercial laboratory. Briefly, soil NH₄⁺ and NO₃⁻ ions were extracted by 2 M KCl solution from fresh soil using a 1:10 soil/solution ratio (w/v) and determined by flow injection analyzer. Soil pH was analyzed by an automated analyzer using a 1:1 soil/water ratio (v:v). Transformation of nitrogen was determined by taking two top 30-cm soil samples from plots treated with 448 kg·ha⁻¹ EDN on a weekly basis for 10 weeks. The top 30 cm of soil was used because any nitrogen deposited below this depth would likely never be used by crop

species. The use rate of 448 kg·ha⁻¹ was chosen based on previous efficacy studies on soil-borne pests. Soil samples were separated into 0–15-cm and 15–30-cm sections for pH, NO₃⁻, and NH₄⁺ ion analysis using the above-described methods. Week 7 data in the Fall 2018 season were missing due to a significant weather event (i.e., Hurricane “Michael”) that impacted the area.

Data were analyzed using mixed model methodology. EDN rate was treated as a fixed effect, while sampling dates and soil depth were treated as double repeated measures. Covariance matrix for sampling date and soil depth was unstructured and first-order autoregressive, respectively. Random effects included season, block, and their interactions with other factors. To test for differences among EDN rates, sampling dates, soil depth, and their interactions, Bonferroni’s post hoc test was performed using SAS 9.4 software at $\alpha = 0.05$ level (SAS Institute Inc., Cary, NC). Figures were composed using SigmaPlot 14.0 software (Systat Software, San Jose, CA).

Results

Nitrogen deposition. Soil pH of the top 0–15-cm soil significantly increased from 6.3–6.4 before fumigation to 7.0–7.1 at 3 weeks after fumigation (WAF). However, fumigation had minimal impacts on pH of the deeper soil profile (i.e., 15–30 and 30–45 cm). No significant differences were observed among the three EDN rates regarding their impacts on soil pH at 3 WAF (Table 2).

Fumigation with EDN notably increased soil NH₄⁺ concentration in top 0–15- and 15–30-cm soils, but not in 30–45-cm soils (Table 2). At 3 WAF, NH₄⁺ concentration showed a significant increase between 448 and 560 kg·ha⁻¹ (34.8 and 40.0 mg·kg⁻¹ soil, respectively) at 0–15 cm, whereas concentrations were similar between 336 and 448 kg·ha⁻¹. A similar trend was observed at 15–30 cm, but the differences among EDN rates did not reach a significant statistical level. No significant differences were detected among EDN rates at 30–45 cm for NH₄⁺ concentration (Table 2).

Soil NO₃⁻ concentration did not significantly change at 3 WAF across all the evaluated soil profiles, relative to the background concentration. No significant differences among EDN rates were observed either, regardless of soil depth (Table 2).

Nitrogen transformation. Soil pH significantly increased from 0 to 1 WAF and remained relatively consistent for the rest of the season at 0–15 cm. The 15–30-cm soils

had notably lower soil pH compared with the top 0–15-cm soil, and fumigation with EDN had minimal effect on soil pH at 15–30 cm (Fig. 1).

Soil NH₄⁺ concentration significantly increased from 2.8 mg·kg⁻¹ at 0 WAF to 38.5 mg·kg⁻¹ at 1 WAF at 0–15 cm. Following the initially sharp increase, NH₄⁺ level continued to slowly increase until reaching its peak concentration at 7 WAF. After that, soil NH₄⁺ concentration started to decline sharply. Soil NH₄⁺ concentration eventually (i.e., 10 WAF) dropped to a level similar to 3–4 WAF (Fig. 2A). A similar trend also applied to soil NH₄⁺ concentration at 15–30 cm (Fig. 2B).

Unlike soil NH₄⁺, soil NO₃⁻ concentration remained relatively consistent from 0 to 7 WAF and linearly increased afterward in both 0–15- and 15–30-cm soils. Compared with 0 WAF, soil NO₃⁻ concentration at 10 WAF significantly increased by 6- and 4-times in 0–15 and 15–30 cm, respectively (Fig. 2A and B). Soil NH₄⁺ and NO₃⁻ may continue to change beyond the duration of the current study.

A comparison of NO₃⁻ and NH₄⁺ at each soil depth was analyzed to determine the effect of EDN on nitrification. Before fumigation (i.e., 0 WAF), no significant differences were observed between NO₃⁻ and NH₄⁺ at both soil depths (Fig. 2A and B). At 0–15 cm, soil NH₄⁺ concentration was consistently greater than soil NO₃⁻ concentration from 1 to 10 WAF (Fig. 2A). In contrast, significant differences between soil NH₄⁺ and NO₃⁻ concentration at 15–30 cm only occurred between 6 and 8 WAF (Fig. 2B).

Discussion

Due to the nonselective nature of soil fumigants, effects on soil microbes are typically observed, including those responsible for transformation of nitrogen. Soil fumigation often results in an increase of NH₄⁺ released from the killed microbial biomass and can remain in an unoxidized state until nitrifying bacteria reestablish sufficient populations to oxidize NH₄⁺ (Jenkinson and Powlson, 1970; Rovira, 1975; Shen et al., 1984).

One objective of this study was to determine deposition of nitrogen by EDN at different rates. It is well known that NH₄⁺ is a degradation product of EDN; therefore, this was done by analyzing average NH₄⁺ concentration for each rate at 3 WAF. We observed NH₄⁺ levels ranging from 42.3–54.9 mg·kg⁻¹ at 3 WAF in soils that had no fertilizer applied before fumigation. Thus it

Table 2. Soil pH, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ in top 0–15-, 15–30-, and 30–45-cm soils immediately before (“Pre”) and 3 weeks after (“Post”) fumigation with ethanedinitrile (EDN). Experiments were conducted in Quincy, FL.

EDN rate (kg·ha ⁻¹)	0–15 cm		15–30 cm		30–45 cm	
	Pre	Post	Pre	Post	Pre	Post
	pH					
336	6.4 a B ^c	7.0 a A	6.0 a A	6.3 a A	6.3 a A	6.1 a A
448	6.4 a B	7.0 a A	6.1 a A	6.3 a A	6.1 a A	6.1 a A
560	6.3 a B	7.1 a A	6.0 a A	6.3 a A	6.1 a A	6.1 a A
<hr/>						
336	1.4 a B	31.5 b A	1.6 a B	8.5 a A	1.5 a A	2.3 a A
448	1.6 a B	34.8 b A	1.4 a B	11.3 a A	1.8 a A	3.2 a A
560	1.4 a B	40.0 a A	1.7 a B	12.2 a A	1.9 a A	2.7 a A
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336	4.9 a A	3.6 a A	4.3 a A	5.1 a A	5.2 a A	3.8 a A
448	3.1 a A	3.7 a A	3.6 a A	3.6 a A	5.1 a A	6.0 a A
560	2.9 a A	2.2 a A	5.6 a A	4.5 a A	7.5 a A	3.8 a A

^aMeans followed by lowercase letters within each column indicate significant differences among EDN rates, while means followed by different uppercase letters within each soil depth indicate significant difference between pre- and postfumigation at $\alpha = 0.05$ level using the Bonferroni method.

Fig. 1. Soil pH in top 0–15- and 15–30-cm soils immediately before and weekly after fumigation with 448 kg·ha⁻¹ ethanedinitrile (EDN). Data represent means $\pm$ SE. Different lowercase letters indicate significant differences among sampling dates for each soil depth.

may be reasonably assumed that the NH_4^+ measured at this time resulted from the application of EDN. There was a significant increase in ammonium deposition between 448 and 560 kg·ha⁻¹ but not between 336 and 448 kg·ha⁻¹, though the percentage increase was similar between both rate increases. Ammonium deposition clearly increases with EDN use rate, but the three rates used in this study are most likely to be deployed commercially because of the pest control efficacy.

Most of the significant effects observed during these experiments were in the top 30 cm of the soil profile; indeed the most profound effects were observed in the top 15 cm. Because of the volatile nature of EDN, it is hard to track its movement within the soil profile. Because of its high vapor pressure, it has been hypothesized that EDN will move extensively in the soil profile. While this may be the case, fumigant vapor will eventually move toward the soil surface, and it may not reside long enough at these depths for complete transformation from the EDN molecule into its byproducts.

Transplanting of vegetable crops typically occurs 3 WAF. At this time, soil $\text{NH}_4^+\text{-N}$ of 40.0 mg·kg⁻¹ (equivalent to ≈ 90 kg·ha⁻¹) was detected at the lowest rate of EDN, which exceeds the recommended rate of preplant N fertilizer for crops such as tomato and pepper (≈ 78.5 kg·ha⁻¹) for Florida production systems (Liu et al., 2020). These data suggest that fumigation with EDN has the potential to eliminate application of preplant N fertilizer for some crops. Meanwhile, use of EDN could result in excessive preplant nitrogen, which could be detrimental for some crops, such as strawberry, be-

cause excess nitrogen can cause the plant to produce too many runners, fewer flowers, and poor-quality fruit (Hochmuth et al., 1996). Average soil NH_4^+ deposited by EDN fumigation was 47.7 mg·kg⁻¹, which could save producers \$38.37 per acre in nitrogen fertilizer costs compared with the use of urea fertilizer (B. Lanier, personal communication).

Nitrification of NH_4^+ is an important step in the nitrogen cycle. In one study, NH_4^+ added to unfumigated soil was nitrified within 1 week, while fumigated soil inhibited nitrification for at least 3 weeks in all evaluated soil types (De Neve et al., 2004). In the current study, inhibition of nitrification occurred for at least 7 weeks. The difference in nitrification inhibition could be due to the slightly different soil types because fumigant movement is highly dependent on soil type and could affect microbial recolonization. A depression of nitrification for 4 months due to fumigation with a dichloropropene-containing fumigant in loamy sand soil was previously reported (Draycott and Last, 1971). Fumigation with the same dichloropropene-containing fumigant on a sandy loam soil depressed nitrification for 3 weeks in another study (Tu, 1993, 1994).

De Neve et al. (2004) reported peak NH_4^+ levels in fumigated soil at 5 WAF, followed by a sharp decline. In the current study, peak NH_4^+ levels occurred 2 weeks later (i.e., 7 WAF) before it started to decrease. This suggests nitrification is inhibited for an increased period in these soils when EDN is applied. In coarse-textured soils, decreased conversion of NH_4^+ to NO_3^- could have a positive impact, because these soils have a high leaching potential. However, nitrification inhibition

Fig. 2. Soil NH₄⁺-N and NO₃⁻-N in top (A) 0–15- and (B) 15–30-cm soils immediately before and weekly after fumigation with 448 kg·ha⁻¹ ethanedinitrile (EDN). Data represent means ± SE. Different lowercase letters indicate significant differences among sampling dates for NH₄⁺-N or NO₃⁻-N, whereas different uppercase letters indicate significant differences between NH₄⁺-N and NO₃⁻-N at each sampling date.

could also have a detrimental impact because high ammonium concentration could cause toxicity or compete for binding sites in the soils with other cationic nutrients (e.g., K⁺), leading to potential nutrient deficiencies.

As also shown in previous studies, fumigation in this study resulted in significant change in nitrification. Fumigation with EDN results in deposition of NH₄⁺; however, the amount of ammonium deposited by EDN is still variable. Transformation of NH₄⁺ to NO₃⁻ was inhibited for at least 7 weeks. This could be impacted by planting a crop, or the addition of irrigation, which will introduce new microbes to the fumigated bed and may speed microbial recolonization.

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