Short communication

Nitrogen starvation promotes biodegradation of N-heterocyclic compounds in soil

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Abstract

Mineralization studies were performed to examine the impact of N deprivation on microbial utilization of the N-heterocyclic herbicides, atrazine and cloransulam-methyl (C-M). Soil depleted by 130 years of cropping to *Zea mays* without fertilization was contrasted to soil from the same site regularly receiving fertilizers. Long-term N deprivation promoted rapid degradation of atrazine and the C-M pyrimidine ring, whereas no significant effect was observed on degradation of the C-M phenyl ring. When a sandy soil naturally low in N was used, addition of 5 or more $\mu g\,NH_4$–N/g soil suppressed mineralization of the C-M pyrimidine ring. These findings provide insight into organic N availability and suggest broad implications for the effect of exogenous N in degradation of heterocyclic herbicides. Published by Elsevier Ltd.

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Soil microbial communities respond to N starvation by scavenging N from protein (Sims and Wander, 2002) and presumably, many other N-containing organic compounds. Simple N sources, such as ammonium, are generally involved in down-regulation of pathways for salvaging N from intracellular pools of costly bio-molecules, such as amino acids (Atkinson and Fisher, 1991), purines (Nygaard et al., 1996), and intracellular proteins (Hemmings, 1978). Similarly, ammonium is preferred over atrazine as an N source by certain bacteria (Cook and Hütter, 1981; Garcia-Gonzalez et al., 2003; Mandelbaum et al., 1995; Radosevich et al., 1995) as well as soil communities (Bichat et al., 1999; Rhine et al., 2003). In addition to the examples given, the soil community is exposed to many complex nitrogenous compounds, including other pesticides. For example, most herbicides targeting photosynthesis, amino acid or lipid biosynthesis, as well as many auxins and safeners, are biodegradable N-heterocycles. Most bear multiple heteroatoms and many contain both heterocyclic and homocyclic ring systems. The propensity of micro-organisms to regulate decomposition of nitrogenous organic materials is well documented, and may be applicable to whole soil communities. Such regulation has broad implications for nutrient, waste, and pest management strategies.

Herein is described a simple experiment that examines the mineralization of two heterocyclic herbicides, atrazine and cloransulam-methyl, under conditions of N starvation in soil. Soils used in the study had either been depleted of N by cropping without fertilizers over an extended time period, or had inherently low inorganic N concentrations. This facilitated low enough background N concentrations to observe response (in mineralization rate) to added inorganic N. Cloransulam-methyl is rapidly deactivated in soil via removal of alkyl groups, however, mineralization of the ring systems proceeds more slowly (Cupples et al., 2000; Wolt et al., 1996), with kinetics similar to those expected for atrazine. Though both herbicides are rich in N, cloransulam-methyl has both hetrocyclic and homocyclic ring systems that can be differentially labeled to facilitate independent measurement of the fates of these moieties. Cloransulm-methyl (applied at rates up to 0.05 kg ai/ha) is used largely for soybean production, in

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which N fertilizers are generally not applied, in contrast to atrazine (applied at rates up to 1.7 kg ai/ha), which is generally used in corn and sorghum, where N fertilizers are typically applied at relatively high rates.

Properties of soils used are detailed in Table 1. The Flanagan silt loam soil (fine, montmorillonitic, mesic Aquic Argiudolls) was sampled from University of Illinois Morrow long-term soil fertility plots (Aref and Wander, 1998) that had received either 0, or 224 kg urea-N/ha (plus 50 kg K/ha as KCl and 377 kg P/ha as $Ca(H_2PO_4)_2$) over a period of >130 years. The unfertilized Plainfield loamy sand (mixed, mesic Typic Udipsamments) was collected from an uncropped area in Kilbourne, IL, and fertility was augmented with 0, 1, 5, 10, or 20 μg NH_4–N/g soil as NH_4SO_4.

^{14}C-mineralization studies were performed in triplicate with 5-g soil samples in biometer devices described previously (Taylor-Lovell et al., 2000). Labeled herbicides (each 97–98% radiochemically pure) were introduced with deionized water used to adjust water content from field moist to 40% of the water holding capacity at 300 kPa. Soil was introduced and biometers were sealed with a lid fitted with a CO_2 trap containing 1 mL of 0.1 M NaOH. Atrazine was supplied at a rate of 1.6 μg herbicide/g soil with UL

ring-labeled material at a specific activity adjusted to deliver 437 Bq/g soil, whereas cloransulam-methyl was introduced at 47 ng/g soil in one treatment as phenyl-UL-^{14}C- and as pyrimidine-7,9-^{14}C-labeled material in another to deliver approximately 300 Bq/g soil in either case. These treatments represented approximate herbicide concentrations expected in the field, assuming a normal application is incorporated to a depth of 7.5 cm by rainfall.

The results in Table 2 show that atrazine and the pyrimidine ring of cloransulam-methyl were more rapidly mineralized in N-depleted than fertilized soil from the Morrow Plots, while N depletion had no detectable effect on mineralization of the phenol ring. Addition of 10 μg NH_4^+–N/g soil resulted in slower N degradation kinetics in the N-depleted soil, but not the soil that had been regularly fertilized (Table 2). When fertilized Flanagan soil had been initially leached with 0.01 M $CaCl_2$ (to deplete nitrate to less than 5 μg NO_3^-–N/g) prior to incubation with a herbicide, increased mineralization was observed with both atrazine and the cloransulam-methyl-pyrimidine ring, whereas no significant increase was observed when the leached soil had undergone long-term depletion or was subsequently fortified with 10 μg NH_4–N/ mL. Leaching had no significant effect on mineralization of the cloransulam-methyl phenyl ring.

In the Plainfield soil, which had an initial inorganic N concentration of <5 μg N/g soil, supplementing with an additional 5 or more μg NH_4^+–N/g soil resulted in significantly slower mineralization of both atrazine and the cloransulam-methyl pyrimidine ring (Fig. 1). No significant effect of added N was observed for cloransulam-methyl labeled in the phenyl ring.

In this study, neither organic substrate was a major source of N. Atrazine provided at most 0.5 μg N/g soil, whereas the N contribution from cloransulam-methyl was potentially 60-fold less, though degradation of both compounds was affected by N status. Analogous findings were recently reported for proteolytic activity, in which a

Table 1
Properties of soils used[a]

Soil	pH	Total N	Organic C	WHC (300 kPa)[b]
		g/kg soil		
Flanagan (unfertilized)[c]	5.4	1.47	15	210
Flanagan (fertilized)	6.2	1.88	16	210
Plainfield	7.7	0.38	3	80

[a]Soils sampled to a depth of 7.5 cm.
[b]WHC = water holding capacity.
[c]Data for Flanagan soil taken from Aref and Wander (1998).

Table 2
Effect of nitrogen deprivation (Flanagan soil) on mineralization of C from the atrazine ring, cloransulam-methyl phenyl ring, and cloransulam–methyl pyrimidine ring

Soil fertilizer history	Unleached			Leached with $CaCl_2$		
	CM-pyrimidine[a]	CM-phenyl[b]	ATR	CM-pyrimidine[a]	CM-phenyl[b]	ATR
	%^{14}C mineralized[c,d]					
Soils Treated with 0 μg NH_4 N/g						
Depleted	24	15	31	25	14	26
Fertilized	9	14	6	18	10	14
Soils treated with 10 μg NH_4 N/g						
Depleted	9	16	22	12	17	19
Fertilized	8	17	7	10	15	8

[a]CM-pyrimidine = cloransulam-methyl with pyrimidine label.
[b]CM-phenyl = cloransulam-methyl with phenyl label.
[c]Cumulative mineralization over a 40-day incubation period.
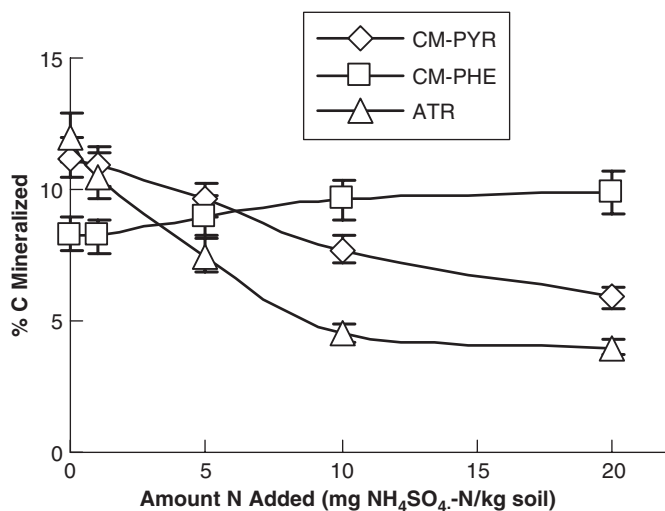[d]Fisher's LSD = 5.48%.

Fig. 1. Mineralization of carbon from the atrazine ring, and the phenyl and pyrimidine rings of cloransulam-methyl as a function of added NH_4SO_4 in a Plainfield sand soil. Error bars indicate one standard deviation. CM–PYR = cloransulam-methyl with a pyrimidine label, CM–PHE = cloransulam-methyl with a phenyl label, ATR = atrazine. Fisher's LSD = 1.06%.

soil community responded to either N or S deficiency with increased proteolytic activity, even though protein available clearly provided insufficient N or S to utilize the carbon present as glucose (Sims and Wander, 2002). Collectively, these results demonstrate that soil microbial communities exhibit some degree of regulation when scavenging traces of N from natural and anthropogenic sources when inorganic N supplies are limited.

Degradation of N-heterocycles is expected to be regulated by N status at the organism level, however, effects of fertilization on degradation are seldom observed at the bulk process level when productive agricultural soils are used (Assaf and Turco, 1994), probably due to significant inorganic N concentrations even when these soils are unfertilized. Actual use of herbicides like atrazine or cloransulam-methyl under the extreme N-depleted conditions described herein is unlikely, as fertility management generally accompanies herbicide use, however, the results provide insight into regulation of N nutrition in microbial communities. These findings however, could be of practical significance for remediation of certain herbicide spills by promoting N immobilization, perhaps by incorporating residues with wide C/N ratios. The results also support the need for continued research into the interactions among agricultural practices, such as fertility and weed management.

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