Biol Fertil Soils (1999) 29:348–353 © Springer-Verlag 1999

ORIGINAL PAPER

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Influence of nitrogen on atrazine and 2, 4 dichlorophenoxyacetic acid mineralization in blackwater and redwater forested wetland soils

Received: 26 June 1998

Abstract Microcosms were used to determine the influence of N additions on active bacterial and fungal biomass, atrazine and dichlorophenoxyacetic acid (2,4-D) mineralization at 5, 10 and 15 weeks in soils from blackwater and redwater wetland forest ecosystems in the northern Florida Panhandle. Active bacterial and fungal biomass was determined by staining techniques combined with direct microscopy. Atrazine and 2,4-D mineralization were measured radiometrically. Treatments were: soil type, (blackwater or redwater forested wetland soils) and N additions (soils amended with the equivalent of 0, 200 or 400 kg N ha^{-1} as NH_4NO_3). Redwater soils contained higher concentrations of C, total N, P, K, Ca, Mn, Fe, B and Zn than blackwater soils. After N addition and 15 weeks of incubation, active bacterial biomass in redwater soils was lower when N was added. Active bacterial biomass in blackwater soils was lower when 400 kg N ha^{-1}, but not when 200 kg N ha^{-1}, was added. Active fungal biomass in blackwater soils was higher when 400 kg N ha^{-1}, but not when 200 kg N ha^{-1}, was added. Active fungal biomass in redwater soils was lower when 200 kg N ha^{-1}, but not when 400 kg N ha^{-1}, was added. After 15 weeks of incubation 2,4-D degradation was higher in redwater wetland soils than in blackwater soils. After 10 and 15 weeks of incubation the addition of 200 or 400 kg N ha^{-1} decreased both atrazine and 2,4-D degradation in redwater soils. The addition of 400 kg N ha^{-1} decreased 2,4-D degradation but not atrazine degradation in blackwater soils after 10 and 15 weeks of incubation. High concentrations of N in surface runoff and groundwater resulting from agricultural operations may have resulted in the accumulation of N in many wetland soils. Large amounts of N accumulating in wetlands may decrease mineralization of toxic agricultural pesticides.

Key words Wetlands · Blackwater · Redwater · Atrazine · 2,4-dichlorophenoxyacetic acid

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Introduction

The chemical characteristics of forested wetland soils on river floodplains in the southeastern United States can be divided into two predominantly different types based on physical and chemical characteristics. Blackwater streams originate in swamp bogs and marshes or drain areas that have nutrient poor soils. These streams have high dissolved organic C concentrations which imparts a dark color to the water and low concentrations of both dissolved and particulate associated inorganic ions (Windom et al. 1971; Beck et al. 1974; Smock and Gilinsky 1992). Redwater streams drain areas that have poorly consolidated soils of marine Pleistocene origin (Grissinger et al. 1982; Mulholland and Lenat 1992) and contain high concentrations of suspended clays and inorganic nutrients (Beck et al. 1974; Bass and Cox 1985).

The recognition that wetlands can improve water quality has led to their use for wastewater filters throughout the world (Lowrance et al. 1984; Johnson 1991; Crutchfield and Leston 1994). In an effort to preserve dwindling wetland resources, scientists may have oversold the ability of wetlands to retain nutrients and consequently, many wetlands are now used as disposal sites for anthropogenic wastes (Johnson 1991). Vegetative growth in forest wetland ecosystems is often limited by N and P. Nutrients that are taken up by plants and microbes are supplied through nutrient mineralization during the litter decomposition process (Koch and

Reddy 1992; Cooper and Brush 1993). In the past few decades, intensive agricultural fertilization and inadequate wastewater treatment have contributed to increasing concentrations of N (Smolen 1981; Magette et al. 1989; Pickett et al. 1992; Hill 1996) and herbicides (Kolpin et al. 1994; Liu et al. 1996; Pasquarell and Boyer 1996) in surface runoff and groundwater which in some cases have resulted in the accumulation of these chemicals in wetlands (Szeto et al. 1990; Spalding et al. 1994; Goolsby et al. 1994; Wan et al. 1994, 1995; Mersie and Seybold 1996).

High concentrations of N have been shown to inhibit atrazine and dichlorophenoxyacetic acid (2,4-D) degradation by some bacteria and fungi (Donnelly et al. 1993). Alvey and Crowley (1995) found that atrazine mineralization in compost-amended agricultural soils was inhibited when fertilized with 500 kg N ha^{-1} as $CaNO_3$. Entry et al. (1993) found that high concentrations of N decreased atrazine and 2,4-D mineralization in grassland soils. Therefore, high concentrations of N may be expected to reduce the degradation of atrazine and 2,4-D in wetland environments (Entry and Emmingham 1996). The objective of this research was to determine the influence of N additions on active bacterial and fungal biomass and atrazine and 2,4-D mineralization in blackwater and redwater forest wetland soils.

Materials and methods

Site descriptions

The top 10 cm of mineral soil was sampled in three blackwater and three redwater forest wetland floodplains in the northern Florida Panhandle. The blackwater river floodplains were on the Withlacoohae, Suwannee and Acullia rivers. The Withlacoohae river floodplain was sampled at Blue Springs, Florida (30° 30′N 83° 15′W). The site supported a forest consisting of live oak (*Quercus virgiana* Mill.), water oak (*Q. nigra* L.), sweetgum (*Liquidambar styracifoila* L.) and slash pine (*Pinus ellotti* L.). The Suwannee river floodplain was sampled at Suwannee River State Park, Florida (30° 27′N 83° 48′W). The site supported a live oak, turkey oak (*Q. laevis* Walt.), post oak (*Q. stellata* Wangenh.), blackgum (*Nyssa sylvatica* Walt. Sarg.) and slash pine forest. The Acullia river floodplain was sampled near Lamont, Florida (30° 22′N 83° 44′W). The site supported a forest growing baldcypress (*Taxodium distichm* L. Rich), water oak, sweetbay (*Gordonia lasianthus* L. Ellis.), blackgum and sweetgum.

The redwater river floodplains were on the Sandy Creek, Chipola and Escambia rivers. The Sandy Creek floodplain was sampled at Ponce de Leon Recreation Area, Florida (30° 40′N 86° 00′W). The site supported a forest consisting of blackgum, gum (*N. aquatica* L.), baldcypress, sweetgum and sweetbay. Most trees were covered with spanish moss (*Tillandsia usneoides* L.). The Chipola river floodplain was sampled at Florida State Caverns Park, Florida (30° 49′N 85° 15′W). The site supported predominantly baldcypress with some blackgum, laurel oak (*Q. laurifolia* Michx.), swamp oak (*Q. lyrata* Walt.) and water oak. Most trees were covered with spanish moss. The Escambia river floodplain was sampled at University of Western Florida Wetlands, in Pensacola, Florida (30° 30′N 87° 30′W). The site supported a forest growing baldcypress, blackgum, water tupelo, swamp oak, water oak and sweetgum trees.

Soil descriptions

The soil on the floodplain of the Withlacoohe river was a sandy, siliceous, thermic, Arenic Hapludult (Howell and Williams 1990). The A layer was approximately 10 cm thick, dark grey with fine granular structure and a pH of 5.0. The soil on the floodplain of the Suwannee river was a sandy, siliceous, thermic, Aquic Hapludult (Howell and Williams 1990). The A layer was dark grey and approximately 18 cm thick. It had a weak, fine granular structure with a pH of 4.8. The soil on the floodplain of the Acullia river was a sandy, siliceous, thermic, Grossarenic Paleaquult (Howell and Williams 1990). The Ap layer was a dark grey, 18-cm-thick layer with a weak, granular structure and a pH of 5.0.

The soil on the floodplain of the Chipola river was a fine, clay, siliceous, nonacid, thermic Typic Fluvaquent (Duffee et al. 1979). The A1 layer was a dark greyish-brown clay-loam 18 cm thick with a granular structure and a pH of 4.5. The soil on the floodplain of the Sandy Creek was a loamy-clay, siliceous, acid, thermic Typic Fluvaquent (Sullivan 1975). The A1 layer was a dark greyish-brown, loamy-clay approximately 18 cm thick with a pH of 4.8. The soil on the floodplain of the Escambia river was a loamy, siliceous, acid, thermic Typic Fluvaquent (Walker et al. 1960). The A1 layer was a reddish-brown, clay-loam approximately 12 cm thick with a pH of 4.8.

Sampling procedures

Three soil samples were collected from the top 10 cm of mineral soil in three separate 1-m^2 areas of each soil type on 16 and 17 January 1995 when the soil temperature was 18–20 °C and the soil moisture content was 50–60% as measured gravimetrically. The nine samples of the top 10 cm of mineral soil were collected from each site and analyzed for nutrients and active bacterial and active fungal biomass. Nine soil samples from each site with N addition were analyzed for atrazine and 2,4-D mineralization. Soil was collected and stored in air-tight and moisture-tight plastic freezer bags at 4 °C and at moisture conditions similar to those in the field. Soil was prepared for microbial testing within 24 h of collection to minimize the effects of storage on microbial activity (West et al.1986).

Soil chemical analysis

Soil moisture was determined gravimetrically after drying soil to a constant weight at 104 °C for 24 h. At the time of collection, three separate readings of soil temperature were taken at each sampling location. Soil pH was determined with a 1:1 paste of soil and water (McLean 1982). Nitrate was determined using the Lachat autoanalyzer (Lachat Quickchem Systems, Milwaukee, Wis.). Total C was estimated by dry ashing at 525 °C and assuming C equal to 50% of loss on ignition (Nelson and Sommers 1982). Nitrogen was determined using a Lachat autoanalyzer (Bremmner and Mulvaney 1982). Extractable P, K, Ca, Mg, Mn, Fe, Cu, B and Zn was determined by extracting a 2-g sample of the top 10 cm of mineral soil with four aliquots of 0.225 M NH_4OAC plus 0.0005 M diethylentri-aminepentaacetic acid (DTPA). The soil was shaken for 7 min, centrifuged at 180 rpm/min and analyzed for the above elements on a Jarrol Ash 9000 inductively coupled plasma spectrometer.

Experimental design

The laboratory experiment was arranged in a randomized block (Kirk 1982). Treatments were: (1) type of forested wetland (blackwater or redwater) and (2) control (0), and the addition of the equivalent of 200 and 400 kg N ha^{-1} to the top 10 cm of soil. Soil types (sites) were considered as blocking variables. The soil (15 g) was assumed to have bulk density of 1.0 g cm^{-3} and was treated with an equivalent dry weight of 0.025 g of NH_4NO_3 cor-

responding to the 200 kg N ha^{-1} or 0.050 g of NH_4NO_3 corresponding to the 400 kg N ha^{-1}. Control soils (no additional N) received 1 ml of distilled, deionized H_2O.

Microbial biomass measurements

Active, but not total, bacterial biomass and fungal biomass were determined for each soil before C and N additions and on each treatment after incubation using methods described by Ingham and Klein (1984). A 1-g soil sample was diluted in 9 ml of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. A 1-ml aliquot was removed and stained with 1 ml of a 20 μg ml–1 fluorescein diacetate (FDA) solution in a 0.2 M phosphate buffer for 3 min. One milliliter of 1.5% agar in a 0.1 M phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and an aliquot placed on a microscope slide containing a cavity of known volume (Ingham and Klein 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by epifluorescent microscopy. Three slides were evaluated from each sample and ten fields per slide were evaluated with phase contrast microscopy for total hyphal length, and three transects were evaluated for FDA-stained (active) hyphal length at ×160 total magnification.

Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stamatiadis et al. 1990). A 1-ml sample of initial soil suspension was diluted to a final dilution in a 0.2-mg soil in 4 ml buffer. The suspension was incubated with 4 ml of filtered INT buffer for 60 min in the dark at 20 °C. Three slides were evaluated for each sample and ten fields per slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers and size of fluorescent bacteria (Lodge and Ingham 1991). Bacterial volume was computed from the number of soil bacteria per gram of soil considering bacterial spheres to be 1 μm in diameter (Jenkinson and Ladd 1981). A carbon to volume conversion factor of 120 μg C mm^{-3} was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content, and a 0.41 μg C/μg bacteria carbon content in the bacterium or fungus (Jenkinson and Ladd 1981).

Herbicide mineralization

Ring-labeled 14C atrazine (purity >99.5%) was donated by Ciba-Geigy (Greensboro, N.C.) and ring-labeled 14C 2,4-D (purity >98%) was purchased from Sigma (St. Louis, Mo.). We dissolved 1 mM of unlabeled atrazine plus 1995 Bq of ring-labeled ^{14}C atrazine in 10 ml of 95% ethanol. In a separate container, we dissolved 1 mM of unlabeled 2,4-D plus 2557 Bq of ring-labeled 2,4-D in 10 ml of 95% ethanol. Each mixture was brought to 100 ml volume with deionized water. We placed 15 g equivalent dry weight fresh soil in a 50-ml test tube, added 1 ml of the diluted pesticide solutions and thoroughly mixed the soil and herbicides.

Test tubes were then sealed with a rubber stopper with one inlet and one outlet port. Air was passed through soda lime to remove CO_2 and then distilled water at a flow rate of approximately 1660 cm^3/min. At 72-h intervals, moist, CO_2-free air was passed into the tube (Edwards 1982). Exit gases containing $^{14}CO_2$ were passed through an air line into a scintillation vial containing 10 ml of 1 M NaOH to trap CO_2. Atrazine and 2,4-D-mineralization were measured at 5, 10 and 15 weeks of incubation. All treatments were incubated at 20 °C. After each of the three incubation periods, vials containing NaOH were replaced. Subsamples (0.5 ml) from each vial containing NaOH were placed in 20-ml scintillation vials, mixed with a 1-ml distilled, deionized H_2O and 17-ml scintillation cocktail (Bio-Safe II, Research Products International, Mount Prospect, Ill.) and counted for 10 min with a Beckman LS 7500 autoscintillation counter.

We ran 1 set of controls and 1 set blank for each set of 27 samples in order to establish background counts. Control soil samples consisted of 15 g equivalent dry weight of three samples of each blackwater and redwater soil with ^{14}C-labeled herbicide. Control soils were autoclaved for 1 h (252 °C, 1.4 kPa). Blanks consisted of a run of the procedure without soil placed in the container. The amount of ^{14}C counts from control and blank samples was not significantly different from background counts. All herbicide mineralization values are, therefore, reported as values above control values. There were nine replications of each treatment for each soil type with N addition.

Statistical analysis

All dependent variables were tested for normal distribution. Data were then analyzed by means of analysis of variance procedures for a randomized block design with Statistical Analysis Systems (SAS Institute 1986). Residuals were equally distributed with constant variances. All values reported are the sample values minus control values. Differences reported throughout are significant at $P \leq 0.05$, as determined by the Least Squares Means test. Because analysis of variance for soil chemicals, active and total fungal and bacterial biomasses, and atrazine and 2,4-D mineralization did not indicate significant differences among sites, only differences among soil types (blackwater and redwater) and seasons are reported here (Snedecor and Cochran 1980; Kirk 1982).

Results

Redwater soils contained higher concentrations of C, total N, P, K, Ca, Mn, Fe, B and Zn than blackwater soils (Table 1). Active bacterial and active fungal biomass in blackwater and redwater wetland soils were not significantly different prior to incubation; active bacterial and active fungal biomass averaged 0.04 and 0.39 μg C g^{-1} soil. N addition decreased active bacterial biomass in both blackwater and redwater wetland soils after 15 weeks. A significant decrease was observed with 400 kg N ha^{-1}, but not with 200 kg N ha^{-1}. Active fungal biomass in blackwater wetland soils was significantly higher when 400 kg N ha^{-1}, but not when 200 kg N ha^{-1}, was added. Active fungal biomass in redwater wetland soils was nominally lower when 200 kg N ha^{-1}, but not when 400 kg N ha^{-1}, was added (Table 2).

After 5 weeks of incubation the addition of 200 or 400 kg N as NH_4NO_3 ha^{-1} did not influence soil pH or atrazine mineralization (data not shown). After 5 weeks of incubation the addition of 400 kg N ha^{-1} reduced 2,4-D mineralization in blackwater wetland soils (Table 2). After 10 weeks of incubation the addition of 200 or 400 kg N ha^{-1} decreased atrazine mineralization in redwater but not blackwater wetland soils. After 10 and 15 weeks of incubation the addition of 200 or 400 kg N ha^{-1} decreased 2,4-D mineralization in both blackwater and redwater wetland soils. The addition of 400 kg N ha^{-1} did not suppress atrazine or 2,4-D mineralization any more than 200 kg N ha^{-1}. After 15 weeks of incubation 2,4-D mineralization was greater in redwater than blackwater wetland soils. Active bacterial biomass, active fungal biomass and N concentration in incubated soils did not correlate with cellulose mineralization in a linear or curvilinear relationship.

Table 1 Carbon and nutrient concentration in the top 10 cm of blackwater and redwater freshwater wetland soils in northern Florida

Soil type	Carbon	N	P	K	Ca	Mg	Mn	Fe	Cu	B	Zn
	%	g element kg^{-1} soil									
Blackwater	1.3 b[a]	112 b	8 b	29 b	368 b	39 a	6 b	83 b	0.6 a	0.4 b	1.6 b
Redwater	5.9 a	496 a	25 a	61 a	988 a	63 a	33 a	216 a	1.1 a	0.8 a	3.6 a

[a] In each column, values followed by the same letter are not significantly different as determined by Least Square Means Test ($P \leq 0.05$)

Table 2 Active bacterial biomass, active fungal biomass, atrazine and dichlorophenoxyacetic acid (*2,4-D*) degradation in blackwater and redwater river flood plains amended with nitrogen

		Microbial biomass[b]		5 weeks		10 weeks		15 weeks	
	N	Bacteria	Fungi	2,4-D	Atrazine	2,4-D	Atrazine	2,4-D	Atrazine
Treatment	kg ha^{-1} [a]	---- μg C g^{-1} soil ----		----------	----------	% CO_2 recovered	----------	----------	----------
Blackwater		3.4 ab[c]	3.9 bc	3.6 b	2.0 b	21.6 a	3.9 c	30.7 b	6.5 c
	200	2.9 bc	4.3 bc	3.5 b	1.9 b	6.2 c	3.8 c	10.7 c	6.6 c
	400	2.0 c	10.1 a	1.7 c	2.0 b	8.0 c	4.0 c	14.2 c	7.0 bc
Redwater	0	4.3 a	4.8 b	5.0 a	2.4 a	26.5 a	5.3 a	62.9 a	9.6 a
	200	2.9 bc	1.6 bc	3.7 b	2.3 a	16.1 b	4.6 b	38.2 b	7.7 b
	400	3.9 ab	4.2 bc	3.8 b	2.4 a	16.2 b	4.5 b	32.7 b	7.6 b

[a] Top 10 cm of mineral soil assuming a bulk density of 1.0
[b] Estimated after 15 weeks of incubation
[c] In each column, values followed by the same letter are not significantly different as determined by the Least Squares Test ($P \leq 0.05$; $n = 27$)

Discussion

In this laboratory study, we found that high N concentrations in both blackwater and redwater forest wetland soils inhibited atrazine and 2,4-D mineralization. Our findings are consistent with those of similar studies in the literature (Behki and Khan 1986; Entry et al. 1994, 1995; Alvey and Crowley 1995). In vitro studies have shown that high N concentrations in media inhibit herbicide mineralization by mycorrhizal fungi (Donnelly et al.1993; Donnelly and Fletcher 1994). Entry et al. (1993) reported that high N concentrations suppressed atrazine and 2,4-D mineralization in grassland soils. Alvey and Crowley (1995) found that atrazine mineralization in arable soils was inhibited in a compost-amended soil when fertilized with 500 kg N ha^{-1} as $CaNO_3$. Entry et al. (1994, 1995) found that atrazine and 2,4-D mineralization was higher in coniferous forest soils that had similar total N concentrations but higher C:N ratio concentrations than deciduous forest soils. In this study, we found that the addition of 400 kg N ha^{-1} reduced active bacterial biomass in both soils. High N concentrations may stimulate fungal activity in soil, but also act to suppress the enzymes catechol 2,3-oxygenase and protocatecuate 2,3-oxygenase which catalyze the degradation of ring structures of aromatic herbicides (Behki and Khan 1986; Erickson and Lee 1989; Aust 1990; Shuichiro et al. 1991).

The herbicides used in this study were labeled only in the ring structure and, therefore, are in conservative numbers compared to those from studies that have examined the disappearance of the herbicide (parent compound) or studies examining the first and second order alteration of side chains attached to the ring structure. In our study side chain degradation of atrazine or 2,4-D may have occurred, but was undetected because we measured the amount of atrazine and 2,4-D degraded to CO_2. The degradation pathways of atrazine and 2,4-D are well known (Giardina et al. 1982; Chaudhry and Chapalamadugu 1991). Atrazine and 2,4-D could have been degraded to a variety of intermediate compounds. Fungi and bacteria that degrade atrazine via dealkylation produce intermediate compounds that are toxic to some plants and soil microorganisms (Behki and Khan 1986; Alvey and Crowley 1995).

Despite the plethora of information on their toxicities to selected animals in the laboratory, the effects of nutrients and agricultural chemicals on wetland function is unpredictable. The extent of pollution damage to wetland ecosystems is usually measured in one of two ways – by the presence or absence of various indicator species or by specific groups of species genera or families of invertebrates called biotic indices. Usually the concentration of the chemical to kill 50% of a specific number of test organisms (indicator species) in a given time in a laboratory setting (LD_{50}) is measured and from such experiments decisions are made as to safe concentrations (Moss 1993). Although field studies are necessary, data presented in this study lead us to

conclude that N deposition in wetland soils may be causing decreased degradation of agricultural herbicides. Measures of the combined effects of pollutants such as N and/or herbicides on ecosystem function in wetlands has not been thoroughly investigated. The combined effects of the many pollutants that are currently being deposited in wetlands may have a greater impact on ecosystem function that those impacts that are measured in the laboratory under specific conditions.

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