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Comparing Carbon Substrates for Denitrification of Subsurface Drainage Water

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ABSTRACT

Nitrate in water from tile drained corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] fields in the U.S. Midwest contributes to nitrate contamination of surface waters. Denitrification-based biofilters are a promising strategy for reducing nitrate concentrations, but these systems require an external carbon supply to sustain denitrification. The ability of four organic materials to serve as carbon substrates for denitrification biofilters was evaluated in this laboratory study. Wood chips, wood chips amended with soybean oil, cornstalks, and cardboard fibers were mixed with subsoil (oxidized till) and incubated anaerobically for 180 d. Periodically, $^{15}NO_3$–N was added to maintain nitrate N concentrations between 10 and 100 mg L^{-1}. All of the materials stimulated NO_3–N removal and the degree of removal from highest to lowest was: cornstalks, cardboard fibers, wood chips with oil, and wood chips alone. Analysis of ^{15}N showed that immobilization and dissimilatory nitrate reduction to ammonium accounted for <4% of NO_3–N removal in all treatments, therefore denitrification was the dominant NO_3–N removal process. Cardboard fibers, wood chips and oil, and wood chips alone did not support as much denitrification as cornstalks, but their rates of NO_3–N removal were steady and would probably continue longer than cornstalks. The addition of soybean oil to wood chips significantly increased denitrification over wood chips alone.

Agricultural sources of nitrate (NO_3) in surface waters from the Midwest are a contributing factor to the hypoxia problem in the Gulf of Mexico (Rabalais et al., 1996). Specifically, subsurface drainage in the Midwest is considered a major source of NO_3 delivered to the Gulf of Mexico. Nitrate nitrogen concentrations leaving subsurface drains in Iowa routinely exceed the USEPA Maximum Contaminant Level for drinking water of 10 mg L^{-1} (Gast et al., 1978; Jaynes et al., 1999). Nitrate is produced from fertilizer N and from soil organic matter. The combination of snowmelt, spring precipitation, NO_3 production in soil, and lack of significant plant uptake result in the leaching and movement of NO_3 into tile drainage networks which eventually reach surface waters (Dinnes et al., 2002).

Microbial denitrification is a process that converts NO_3 to nitrogen gases (N_2 and N_2O), but in subsurface soils it is generally limited by available organic carbon C (McCarty and Bremner, 1992; Cambardella et al., 1999; Richards and Webster, 1999). Cambardella et al. (1999) incubated subsurface soil, oxidized glacial till, and unoxidized glacial till with NO_3 and observed that <0.3 mg NO_3–N g^{-1} material was removed over 1070 d. When these materials were incubated with NO_3 and glucose, denitrification rates increased tenfold.

Several denitrification-based strategies have emerged for reducing the NO_3 load in tile drainage water. All of these strategies rely on creating a treatment zone where drainage water passes through an area amended with an organic carbon source that stimulates NO_3 removal through denitrification. Several investigators have constructed denitrification walls to intercept NO_3–contaminated ground water. Schipper and Vojvodic-Vukovic (1998, 2000, 2001) reported on a wall consisting of an excavated trench filled with soil mixed with sawdust to provide C for denitrification. As ground water moved through the wall, microbial denitrification removed the NO_3. This wall successfully reduced subsurface water NO_3 concentrations from 5 to 16 mg NO_3–N L^{-1} to <2 mg NO_3–N L^{-1}. Robertson et al. (2000) described a similar system built to intercept septic-system effluent. We have also constructed denitrification walls that remove NO_3 from subsurface drainage water leaving agricultural fields in Iowa (Jaynes et al., 2004). Blowes et al. (1994) described a bioreactor filled with wood chips, tree bark shavings, and compost that was placed at the end of a tile-line where it effectively removed nitrate from drainage water.

Despite the apparent short-term success of denitrification walls and bioreactors in stimulating denitrification, factors such as the type, quantity, and particle size of organic amendments on the rates of denitrification remain poorly understood. Denitrification rates need to be sufficiently stimulated by the carbon amendments to effectively remove the bulk of the NO_3. Furthermore, the length of time that a potential carbon substrate can support denitrification is also critical to in situ treatment of drainage water. Previous studies with denitrification walls have used different carbon substrates with a range of particle size (from sawdust to compost to wood bark). While other studies have shown that a wide variety of oils, sugars, organic acids, and alcohols can stimulate denitrification (Obenhuber and Lowrance, 1991; Hunter et al., 1997; Smith et al., 2001) comparative information on solid substrates is lacking.

Finally, previous studies have not evaluated the pathways and fate of the NO_3 removed from the drainage water by in situ biofilters. Denitrification is commonly reported as the mechanism of NO_3 removal. Increased activity of denitrification enzymes (denitrifying enzyme assay) provides evidence for denitrification in some of these studies (Schipper and Vojvodic-Vukovic, 2000), but nitrogen immobilization and dissimilatory nitrate reduction to ammonia (DNRA) may also be important mechanisms of NO_3 removal in anaerobic systems (Tiedje, 1988). If substantial amounts of NO_3 were converted to

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Abbreviations: DNRA, dissimilatory nitrate reduction to ammonia.

NH_4 or organic N rather than N_2, then N may be exported from the system in that form, or remain in the system and later be converted to NO_3 thus defeating the treatment strategy.

Thus, our first objective was to investigate the effectiveness of different C sources to stimulate denitrification in laboratory microcosms. We also ground these materials to investigate the effect of particle size on NO_3 removal. Our second objective was to determine the contributions of denitrification, dissimilatory NO_3 reduction to NH_4, and N immobilization to decreases in NO_3 concentrations.

MATERIALS AND METHODS

The four different C sources used in this study included: (i) wood chips (predominately *Quercus* spp.) approximately 3 to 10 cm in length, (ii) wood chips saturated with soybean oil (48% oil by weight), (iii) dried cornstalks collected after harvest, and (iv) paper fibers from corrugated cardboard. The C and N contents of these materials are listed in Table 1. These same carbon sources were ground to <2-mm size to determine what effect particle size would have on rate or mechanisms of NO_3 removal. The cardboard fibers were similar to those described by Kruse (1995) and were collected as a dried slurry derived from pulped corrugated containers at a recycle mill.

The influence of these carbon sources on denitrification was determined in anaerobic incubations where 5 g carbon material (dry weight basis) was mixed with 5 g of field moist subsoil and 149 mL distilled water in 180-mL glass jars. The 1:1 ratio (wet soil to dry carbon material) resembles conditions in a denitrification wall under field conditions. The final volume of liquid in the incubation microcosms was increased over that found in the field to submerge all of the carbon source materials used in the experiment.

All jars received 1 mL of a $^{15}NO_3$–N solution that contained 15 mg NO_3–N mL^{-1} (10 atom % ^{15}N) (Isotec, Miamisburg, OH). The soil used to inoculate the microcosms was oxidized till from the Des Moines Lobe glacial formation (Eidem et al., 1999) taken 2 m below the surface of a Canisteo series soil (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) located in a corn–soybean-rotated field near Boone, Iowa (C and N content listed in Table 1). Jars prepared as described above without any carbon source (subsoil only) served as the control. Jars were incubated for 180 d at 20 ± 2°C in an anaerobic growth chamber with a N_2 gas atmosphere. Oxygen content in the chamber was monitored daily and kept at <1% by evacuating and purging the chamber with N_2 as necessary. Temperature was monitored hourly with a thermocouple and a datalogger. A sufficient number of jars (162) were prepared so that three replications could be destructively sampled on six dates spaced 30 d apart.

Throughout the experiment NO_3–N concentrations in the jars were monitored every 2 to 7 d and re-treated with 1 mL of the NO_3 solution (15 mg NO_3–N mg mL^{-1} with 10 atom % ^{15}N) when solution concentrations were <10 mg L^{-1}. Jars were briefly shaken 1 h before a sample was taken and immediately after the NO_3–N solution was added. At 30-d intervals, three replicates of each treatment were sacrificed for aqueous-phase analysis of NO_3–N and NH_4–N, isotope ratios, and dissolved organic carbon (DOC), as well as solid-phase analysis of total N, ^{15}N, and organic C. Jar contents were mixed thoroughly and poured into 180 mL high-density polyethylene bottles, then centrifuged for 30 min at 576 × *g* at 8°C. The supernatant was decanted, filtered, and used for analysis. The remaining soil and organic amendment were rinsed two more times to remove any remaining NO_3–N and NH_4–N in the moist pellet by adding 100 mL distilled water, mixing thoroughly, and centrifuging as described above. These rinse water supernatants were discarded. The rinsed solid materials were dried at 60°C for 72 h.

Table 1. Organic C and N contents of oxidized till and C sources used as amendments.

	Organic C	Total N	C to N ratio
	g kg^{-1}		
Oxidized till	2.1	0.3	7.0
Cornstalks	404.5	9.5	42.6
Cardboard fibers	420.4	1.5	280.3
Wood chips and soybean oil	636.5	0.8	795.6
Wood chips	493.8	1.1	448.9

Dried solid materials were ground to <2 mm using a Wiley mill then further ground to a fine powder by using a Cyclone mill. Organic C and N contents in the ground samples were determined by combustion using a Carlo Erba (Milan, Italy) NA1500 NSC elemental analyzer after acid treatment to remove carbonates. For ^{15}N determination, samples were combusted in the elemental analyzer, which was connected to an isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany).

Water extracts were analyzed for NO_3–N (NO_3–N + NO_2–N) and NH_4–N on a Lachat (Milwaukee, WI) autoanalyzer using the colorimetric reaction described by Keeney and Nelson (1982). Ammonium–^{15}N and NO_3–^{15}N were determined by the microdiffusion technique described by Brooks et al. (1989). Dissolved organic carbon contained in water extracts was determined via a Dohrmann DC-180 analyzer (Tekmar-Teledyne, Mason, OH). The pH was measured at each 30-d period using a pH electrode.

The N loss due to denitrification was based on the quantity of NO_3–N removed from solution after accounting for losses due to DNRA and N immobilization. Nitrogen removal (loss from solution) resulting from additions of C source was calculated as the total NO_3–N added minus the residual NO_3–N at the end of the experiment. Dissimilatory NO_3–N reduction to NH_4–N was based on the quantity of ^{15}N appearing as $^{15}NH_4$–N in solution after $^{15}NO_3$–N was added. Nitrogen immobilization was determined by measuring the increase in ^{15}N in the solid material above background (0.366 atom %) as described by Mosier and Schimel (1993) and was assumed to be due to microbial assimilation of ^{15}N. Mass balance estimates of this type have previously been used to estimate denitrification (Rolston et al., 1979).

RESULTS

All carbon substrates stimulated NO_3–N loss from the microcosms (Fig. 1). The NO_3–N loss represents the difference between the total NO_3–N added to the microcosms and the NO_3–N recovered at each sampling date. Cumulative NO_3–N loss was in order of magnitude corn stalks > cardboard > wood and oil > wood. Grinding significantly increased N removal for cardboard and wood at some of the sampling dates. In the cardboard treatment, the grinding effect was evident at Day 60 and persisted up to Day 150. At Day 180, the ground cardboard treatment had a cumulative NO_3–N loss that was lower than the Day 150 measurement and thus grinding was not significantly different from the unground material at Day 180. Increased N loss due to

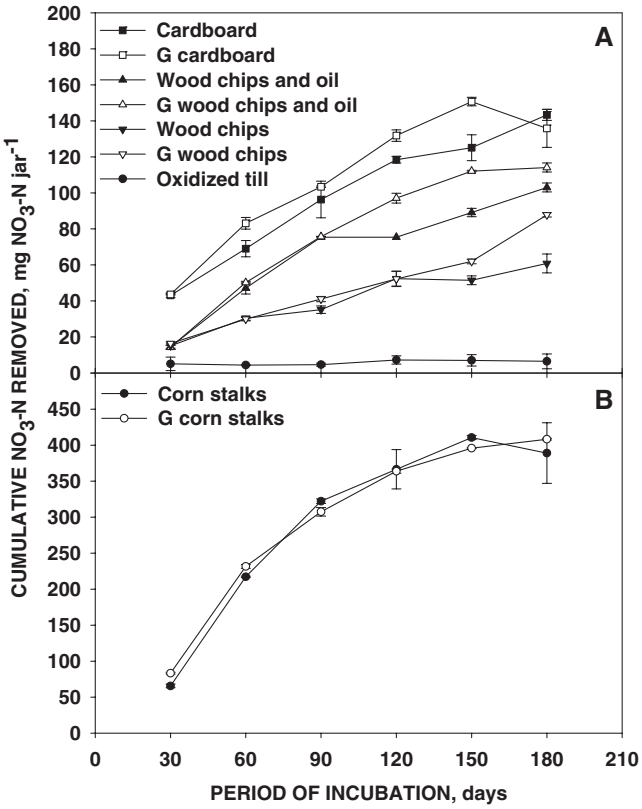

Fig. 1. Cumulative NO_3–N removed measured in 30-d increments from jars incubated with till alone or when amended with a carbon material under NO_3–N saturated conditions. Values are the mean ± standard deviation of three replicate jars. G indicates ground materials. Note the different scales in A and B.

grinding was not evident until Day 120 in the wood chip and soybean oil treatment, and Day 150 in the wood chip alone treatment. The most rapid and greatest total reductions of NO_3–N were in the cornstalk treatments, but there was no significant effect of grinding cornstalks on cumulative NO_3–N removed (Fig. 1B). Nitrate loss in the cornstalk treatments was initially rapid, but the rate decreased steadily throughout the incubation such that at Day 180 the rate of NO_3–N loss was nearly zero (Fig. 1B). Similarly, the ground wood + oil treatment showed evidence of diminished removal rate starting at Day 150. Oil additions to wood chips stimulated N loss compared to wood chips alone at all sampling dates after Day 30 (Fig. 1A). The effect that soybean oil had on the wood started to decline after Day 90 whereas the rate of N loss in the ground wood treatment continued to increase.

The distribution of N in the jar microcosms at the end of the 180-d incubation is shown in Table 2. Nitrate removal by oxidized till without added C was small relative to till with C additions. After 180 d the NO_3–N concentration averaged 9 mg N jar^{-1} in the unamended till. The initial organic N content of the oxidized till treatment averaged 2.2 mg N jar^{-1} and by the end of the incubation declined to 1.8 mg N jar^{-1}. Thus, in this treatment, total N removed over the 180-d period averaged 6.4 mg N jar^{-1}. Greater N losses were observed in the treatments with carbon amendments (Table 2). The greatest losses were observed in the corn stalk treatment, where N loss averaged 389 and 408 mg N jar^{-1} for the un-ground and ground treatments, respectively. Nitrogen losses in the cardboard amendments were nearly threefold less than in the cornstalk treatments and smaller N losses were noted in the woodchip treatments.

Most of the N lost during these incubations came from added NO_3–N, as net changes in the organic N pools were small relative to the amounts of N not recovered (Table 2). The cornstalk treatments showed the greatest loss from the organic pool declining from 49.1 mg N jar^{-1} to approximately 26.5 mg N jar^{-1} after 180 d. However, this decrease represents only 5.6 to 5.9% of the total N lost in this treatment.

Ammonium production was observed in all of the C substrate treatments except the woodchips. Cornstalk treatments supported the greatest increases in NH_4, but total NH_4 production was low relative to the mass of N removed (Table 2). Total NH_4–N production in the cornstalk and ground cornstalk treatments (4.8 and 5.2. mg N) was less than N loss from the organic N pool (22.6 and 23.8 mg N). Mineralization of the organic N under anaerobic conditions should have resulted in corresponding increases in NH_4–N. This apparent loss of N could be due to incomplete extraction of NH_4–N, unmeasured dissolved organic N in the extracts, or volatilization of NH_4–N. Additional samples of cornstalks and till prepared in the same way, but without added nitrate and

Table 2. Nitrogen additions and recovery after a 180-d anaerobic incubation of oxidized till amended with different organic materials.

	Nitrogen added				Nitrogen recovered†				N removed‡
Material	NO_3–N	NH_4–N	Organic N	Total	NO_3–N	NH_4–N	Organic N	Total	Total
	mg N jar^{-1}								
Oxidized till	15.0	0.0	2.2	17.2	9.0 (3.8)	BD§	1.8 (0.4)	10.8	6.4 (4.1)
Cornstalks	405.0	0.0	49.1	453.8	33.4 (45.4)	4.8 (2.5)	26.5 (2.2)	64.8	389.0 (42.0)
Ground cornstalks	390.0	0.0	49.1	438.8	BD	5.2 (0.4)	25.3 (1.4)	30.5	408.6 (1.2)
Cardboard fibers	150.0	0.0	7.8	158.8	6.3 (2.8)	0.5 (0.2)	8.6 (0.5)	15.5	142.3 (3.1)
Ground cardboard fibers	165.0	0.0	7.8	173.8	28.6 (9.3)	1.5 (0.3)	7.8 (1.1)	38.0	134.8 (10.5)
Wood chips	75.0	0.0	6.6	81.6	14.9 (5.2)	BD	5.9 (0.1)	20.8	60.8 (5.2)
Ground wood chips	90.0	0.0	6.6	96.6	3.2 (1.5)	BD	5.6 (0.6)	8.8	87.8 (1.2)
Wood chips and soybean oil	120.0	0.0	5.2	125.3	17.5 (2.3)	0.1 (0.1)	4.7 (0.1)	22.2	103.0 (2.4)
Ground wood chips and soybean oil	135.0	0.0	5.2	140.3	22.3 (2.7)	0.6 (0.2)	3.9 (0.2)	26.2	114 (2.5)

† Values are means of three replicate samples with the standard deviations in parentheses.
‡ Calculated as the difference between total N added and total N recovered.
§ Below detection limit of 0.3 mg L^{-1} for NO_3–N and 1 mg L^{-1} for NH_4–N.

Table 3. Nitrogen-15 additions and recovery after a 180-d anaerobic incubation of oxidized till amended with different organic materials.

Material	^{15}N added†				^{15}N recovered				^{15}N loss
	NO_3–N	NH_4–N	Organic N	Total	NO_3–N	NH_4–N	Organic N	Total	Total
	mg ^{15}N jar^{-1}								
Oxidized till	1.5	0.0	0.01 (0.00)	1.5	0.9 (0.4)	BD‡	0.0 (0.0)	0.9 (0.4)	0.6 (0.4)
Cornstalks	40.5	0.0	0.18 (0.01)	40.7	3.3 (4.5)	0.17 (0.11)	0.6 (0.2)	4.1 (4.7)	36.6 (4.7)
Ground cornstalks	39.0	0.0	0.18 (0.01)	39.2	BD	0.21 (0.02)	0.5 (0.1)	0.7 (0.1)	38.5 (0.1)
Cardboard fibers	15.0	0.0	0.03 (0.01)	15.0	0.6 (0.3)	0.04 (0.02)	0.4 (0.0)	1.0 (0.3)	14.0 (0.3)
Ground cardboard fibers	16.5	0.0	0.03 (0.01)	16.5	2.9 (0.9)	0.12 (0.02)	0.4 (0.1)	3.3 (1.0)	13.2 (1.0)
Wood chips	7.5	0.0	0.02 (0.00)	7.5	1.5 (0.5)	BD	0.1 (0.0)	1.6 (0.5)	5.9 (0.5)
Ground wood chips	9.0	0.0	0.02 (0.00)	9.0	0.3 (0.2)	BD	0.1 (0.0)	0.5 (0.1)	8.6 (0.1)
Wood chips and soybean oil	12.0	0.0	0.02 (0.00)	12.0	1.7 (0.2)	0.01 (0.01)	0.2 (0.0)	1.9 (0.2)	10.1 (0.2)
Ground wood chips and soybean oil	13.5	0.0	0.02 (0.00)	13.5	2.2 (0.3)	0.05 (0.01)	0.1 (0.0)	2.4 (0.3)	11.1 (0.3)

† The ^{15}N in till and organic amendments was estimated using a background of 0.3663 atom % (Mosier and Schimel, 1993). All values shown are means with standard deviations in parentheses.
‡ Below detection limit of 0.3 mg L^{-1} for NO_3–N and NH_4–N.

incubation, showed that approximately 20 mg N jar^{-1} was removed from the cornstalks in the extraction process (data not shown). Extraction losses of N were much lower for the other treatments than for cornstalks, ranging from 0.6 to 1.7 mg N jar^{-1}. Dissolved organic C accounted for 58 and 71 mg C jar^{-1} for the corn and ground corn treatments, respectively (data not shown), which supports the suggestion that some dissolved organic N was lost in the extraction process.

Labeled N (^{15}N) was used to assess the transformation of added nitrate into the organic and NH_4 pools (Table 3). In the unamended till at 180 d, 0.9 mg of the 1.5 mg added ^{15}N was recovered as $^{15}NO_3$–N and ^{15}N above background was not detected as organic N or NH_4. In the organic amendment treatments 79 to 99% of the added ^{15}N was not recovered. Recovery of ^{15}N generally matched the recovery of total N (Table 2). In the treatments with added C, slight increases in the ^{15}N content of the organic N and ammonium pools were observed. The $^{15}NH_4$–N produced in the presence of cornstalks, cardboard fibers, and wood and oil represented only 0.1 to 0.7% of the $^{15}NO_3$–N that was added. Organic ^{15}N was formed in all treatments where a carbon source was added and accounted for 0.8 to 2.5% of $^{15}NO_3$–N that was added.

Data in Tables 2 and 3 were combined to quantify the fate of added NO_3–N according to the processes of denitrification, dissimilatory nitrate reduction to ammonia (DNRA), and immobilization (Table 4). Nitrogen-15 recovered in the organic N pool was used to calculate N immobilization resulting from microbial growth and $^{15}NH_4$–N production was assumed to result from DNRA. By accounting for these processes we can estimate the denitrified N as the remainder of the N not recovered. Treatment differences were determined by a one-way analysis of variance (significant at $P \leq 0.05$) followed by the least significant difference test. In all treatments, denitrification exceeded 98% except for the cardboard, and ground cardboard where denitrification accounted for >96% of the NO_3 transformed. Immobilization accounted for less than 2.4% of the NO_3 transformed in all treatments and DNRA accounted for less than 1% of the NO_3 transformed in all treatments.

DISCUSSION

In situ denitrification barriers and bioreactors have demonstrated nitrate removal from drainage water or shallow ground water. Both Robertson et al. (2000) and Schipper and Vojvodic-Vukovic (1998, 2000, 2001) utilized sawdust to stimulate nitrate removal, while the Blowes et al. (1994) bioreactor used wood chips, wood bark, and leaf compost. Hunter et al. (1997) used soybean oil to obtain nitrate removal. All these strategies for in situ removal of nitrate from ground water have as their underlying rational a stimulation of microbial denitrification through the addition of carbon substrate. While evidence exists that denitrification is indeed stimulated in these systems, it was not determined how much of the nitrate removed was the result of denitrification.

Table 4. Effect of organic amendments on the fate of added nitrate.

Material	NO_3–N added	Fate of added NO_3–N†			
		Total NO_3–N loss	Denitrification	Immobilization	DNRA‡
	mg N jar^{-1}				
Oxidized till	15.0	6.0 (3.8)	5.9 (3.8) f	0.1 (0.0) e	BD§
Cornstalks	405.0	371.6 (45.4)	365.6 (47.0) a	4.2 (2.1) a	1.8 (1.2) ab
Ground cornstalks	390.0	390.0 (0.0)	384.6 (0.7) a	3.2 (0.6) ab	2.2 (0.2) a
Cardboard fibers	150.0	143.7 (2.8)	139.9 (3.0) b	3.3 (0.3) bc	0.5 (0.2) c
Ground cardboard fibers	165.0	136.4 (9.3)	131.8 (10.1) b	3.3 (0.8) bc	1.3 (0.2) b
Wood chips	75.0	60.1 (5.2)	59.0 (5.4) e	1.0 (0.3) ed	BD
Ground wood chips	90.0	86.8 (1.5)	85.7 (1.2) de	1.1 (0.2) ed	BD
Wood chips and soybean oil	120.0	102.5 (2.3)	101.2 (2.3) d	1.3 (0.0) d	0.1 (0.1) c
Ground wood chips and soybean oil	135.0	112.7 (2.7)	111.0 (2.5) cd	1.2 (0.1) ed	0.5 (0.1) c

† Values are the means of three replicates with standard deviations in parentheses. Means followed by different letters are significantly different ($P \leq 0.05$).
‡ Dissimilatory nitrate reduction to ammonium.
§ Below detection limit of 0.3 mg L^{-1} for NH_4–N.

In anaerobic environments there are several possible fates of NO_3, including: assimilatory nitrate reduction (immobilization), DNRA, and denitrification (Tiedje, 1988). The terminal end products of denitrification are gaseous (N_2 and N_2O) and do not contribute to eutrophication. However, NO_3–N transformed to organic N or ammonium through the processes of immobilization and DNRA is not removed from the system. These forms of N remain in the system, and may be subsequently mineralized and/or nitrified to NO_3 if redox conditions change. Anaerobic environments with high C to electron acceptor ratios, such as the bovine rumen, digested sludge, or some estuarine sediments tend to favor DNRA over denitrification (Tiedje et al., 1982). However, precise information on critical C to electron acceptor ratios that control partitioning between these two processes is not yet available. In our incubations, the C to electron acceptor ratio was apparently not sufficiently high enough to favor DNRA over denitrification, as DNRA accounted for <4% of the NO_3–N removed over our range of treatments.

All of the C substrates stimulated denitrification with cornstalks supporting the greatest denitrification, followed by cardboard fibers, wood chips with oil, and wood chips alone. Averaged over the 180-d period, rates of denitrification ranged from 0.427 g N kg^{-1} substrate d^{-1} for the ground cornstalks to 0.066 g N kg^{-1} substrate d^{-1} for the wood chips. This range of rates probably reflects the different amendments' resistance to decomposition with the cornstalks having a lower C to N ratio and less lignification than the wood. Rates in this study are less than those reported by Volokita et al. (1996) for a denitrifying column using shredded newspaper as a C source (0.660 to 0.875 g N kg^{-1} substrate d^{-1}). Cardboard fibers, wood chips and oil, and wood chips did not support as much denitrification as cornstalks, but their rates of NO_3–N removal were still steady at the end of the incubation, while N removal by the cornstalks appeared to have slowed (Fig. 1). The addition of soybean oil, a relatively labile substrate, to the wood chips resulted in increased amounts of denitrification, but the effect tended to diminish by Day 180, suggesting that the soybean oil had been exhausted. Increasing the surface area by grinding the C substrates had inconsistent effects on denitrification and nitrate removal. Differences between ground versus unground cornstalks were not significant, but grinding increased the cumulative N loss for the other substrates at some sampling dates.

Our study was designed to evaluate some materials that could be used in denitrification walls or bioreactors to remove nitrate from tile drainage waters. The choice of materials included solid materials that are relatively inexpensive and widely available. All amendments were effective in increasing denitrification in an incubation experiment where nitrate was generally nonlimiting. Amounts of N immobilized were relatively small and some ammonium accumulated in the cornstalk treatments. This ammonium was derived from the cornstalks and not the added nitrate. Differences in materials should be taken into account in the design of denitrification walls or bioreactors for nitrate removal.

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