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Concentrations and isotope ratios of dissolved inorganic carbon in denitrifying environments

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Abstract. We measured the concentration and isotope ratio of dissolved inorganic carbon (DIC) in groundwater associated with denitrification ($C_{org} + NO_3^- = CO_2 + N_2$) in an agriculturally impacted site in southwestern Michigan. Samples with the lowest nitrate levels also had low dissolved oxygen content and were more depleted in $\delta^{13}C$ than background groundwater. All the samples had DIC concentrations in excess of titratable alkalinity. The magnitude of this DIC in excess of alkalinity correlated with a decreasing $\delta^{13}C$ attesting to the presence of CO_2 derived from organic carbon. Carbon dioxide is one of the by-products of denitrification. Since the carbon isotope ratio of carbon dioxide produced during denitrification can be expected to be similar to that of the substrate consumed in the process, this can serve as a useful tool in monitoring denitrification. The estimated carbon isotope ratio of source carbon added to the DIC pool via denitrification was close to the value obtained for the organic carbon in the aquifer solids, suggesting that the carbon substrate used in denitrification came from soil organic matter. The results provide support for the hypothesis that the concentration and carbon isotope ratios ($\delta^{13}C$) of dissolved inorganic carbon (DIC) in groundwater might be useful to monitor denitrification.

Introduction

Excess concentrations of nitrates in groundwater pose serious health hazards in many regions of the world (U.S. EPA, 1976; S.D.W. Committee, 1977). Nitrate levels beyond those judged to be permissible by the World Health Organization are often found in agriculturally impacted areas, presumably introduced by the addition of artificial fertilizers (WHO, 1984). Denitrification, the bacterially mediated process by which nitrates are converted to nitrogen gas through one of several chemical reactions, is often a natural means by which groundwater is stripped of nitrates. Stable nitrogen isotopes and to a lesser extent those of oxygen and sulfur have been used to monitor this important biogeochemical process (Heaton, 1985; Bottcher et. al., 1990; Komor et. al., 1993). The objective of the present study was to test the hypothesis that the concentration and $\delta^{13}C$ of dissolved inorganic carbon (DIC) in groundwater might be a useful technique to monitor denitrification. For this an agriculturally impacted site in southwest Michigan, USA was chosen.

Sample location, geology and hydrology

The study site, located in Cass County in SW Michigan (41° 55' N, 85° 53' W), is an agriculturally impacted area (Fig. 1). The area is underlain by 60 to 120 m thick calcareous glacial drift consisting of interbedded sands, gravels and clay/till layers (Passero et al., 1981). Regional topography slopes to the south-southwest. Locally, the study area topography is undulating to steep with a 10 m relief and 6 to 18° slopes. Soils occupying the upland and slopes are generally clayey-to-sandy loams (Bowman, 1991). The prominent depressions in the study area were previously occupied by wetlands and thus are more likely histosols (LoVetere, Unpublished data, 1996).

The shallow unconfined aquifer has a saturated thickness of less than 2.5 m above a till layer in the study area (Fig. 1). Depths to water table range from 9 m to less than 2.5 m below the surface. Shallow groundwater in the study area is recharged from rainfall and snow melt. The two topographic depressions at the site collect runoff from the fields and may serve as points for enhanced aquifer recharge and the regional groundwater flow is to the southwest. Groundwater at the study site is a calcium-magnesium-bicarbonate geochemical type.

The main crops cultivated at the site are corn and alfalfa, heavily fertilized by synthetic nitrogen fertilizers and hog manure. Since several of the groundwaters adjacent to the corn fields contain nitrates in excess of permissible levels, this site has been the focus of detailed investigation (Ervin, 1988; Ervin and Lusch, 1992; LoVetere, unpublished data, 1996).

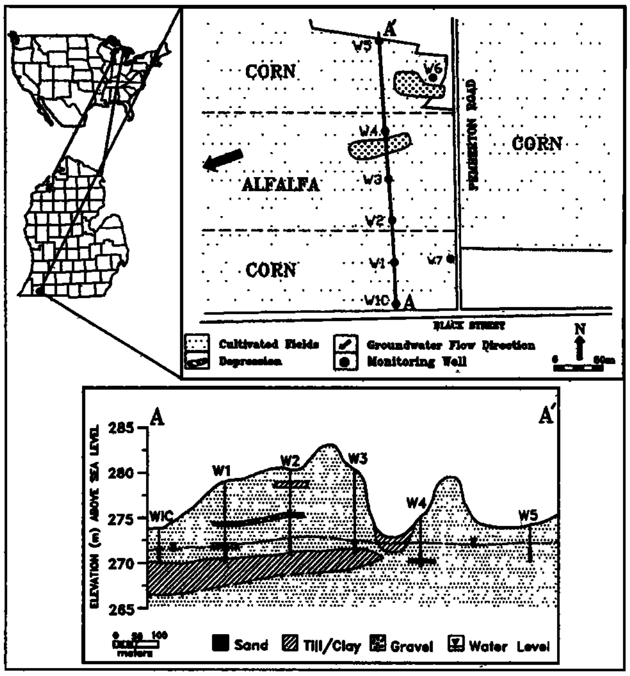

Figure 1. Map of United States showing the study site in Cass County, SW Michigan. Also shown in the figure are the location of wells and a schematic of the geologic cross-section (Modified after LoVetere, Unpublished data, 1996)

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Table 1. Chemical and Isotopic Data of Groundwater from Cass County, SW Michigan

Well	DIC$^{(1)}$	Alk.$^{(1)}$	$\delta^{13}C$ $(^0/_{00})$	$NO_3^{-(2)}$	pH	Mg$^{(2)}$	Ca$^{(2)}$	DO$^{(2)}$	% ex-$_{CO2}$	% DC$^{(3)}$
April 1996										
W1C	624	280	-8.5	47.50	6.81	31.3	154	10.5	122.9	0.04
W1	732	317	-9.7	36.20	6.93	33.3	125	11.3	130.9	0.13
W2	748	349	-14.4	12.28	7.12	32.8	96.3	11.0	114.3	47
W3	776	365	-15.3	3.33	6.95	29.5	102	11.5	112.6	54
W4	398	100	-21.5	0.094	6.03	7.64	19.7	0.3	298	99
W5	268	139	-8.8	34.3	7.33	22.5	69.6	8.3	92.8	0.06
W6	442	127	-17.6	0.186	6.09	11.7	28.6	0.4	248	71
W7	534	260	-14.2	1.39	7.03	23.5	91.9	9.2	105.4	46
July 1996										
W1C	516	211	- 7.9	41.8	7.01	nd	nd	nd	144.5	0.00
W1	746	291	-9.6	41.7	6.84	nd	nd	nd	156.4	0.12
W2	666	293	-15.1	2.51	7.18	nd	nd	nd	127.3	52
W3	796	323	-13.8	3.22	6.95	nd	nd	nd	146.4	43
W4	460	118	-21.1	1.24	6.20	nd	nd	nd	289.8	96
W5	374	177	-9.1	30.3	7.16	nd	nd	nd	111.3	0.08
W6	616	165	-17.1	1.24	6.08	nd	nd	nd	273.3	67
W7	488	193	-15.4	3.16	7.02	nd	nd	nd	152.8	54

$^{(1)}$ = mg/L ($CaCO_3$) $^{(2)}$ = (mg/L)   % DC$^{(3)}$ = % Carbon added via denitrification   nd= not determined

$\delta^{13}C$ of organic $(^0/_{00})$: W1: -22.6; W4: -25.3; W5: -25.8; W6: -24.7; W7: -23.3;   Mean: -24.3 ± 1.4
$\delta^{13}C$ of aquifer carbonates   +1.5 ± $1^0/_{00}$
$\delta^{13}C$ of corn tissue -12.1$^0/_{00}$

Sample collection and analytical methods

Groundwater samples from several wells were analyzed for DIC, stable carbon isotope ratios and other chemical parameters (Fig. 1). Wells were constructed of 5.1 cm diameter (PVC) pipe with a 1.52 m 10-slot PVC screen. Prior to water sampling, three to five pore volumes of water were evacuated from each well with a submersible pump. For DIC analysis, 5 ml of water were collected directly into a syringe and injected into a pre-evacuated septum tube containing phosphoric acid and a magnetic stir bar. Laboratory analysis for DIC involved extracting and measuring the volume of CO_2 from the septum tube under vacuum at 50^0 C while stirring for 10 minutes (Atekwana and Krishnamurthy, unpublished data, 1997). Alkalinity was measured in the field by titration with sulfuric acid using a Hach Alkalinity Kit (HACH, 1992). In addition to water samples, aquifer solids were collected about 0.5 m from each well. These were collected at depths of 20 to 30 cm below ground level and were analysed for the $\delta^{13}C$ values of both the inorganic and organic carbon fractions. All the $\delta^{13}C$ values are reported with respect to PDB. DIC concentrations are expressed as "mg/L $CaCO_3$", based on the CO_2 yield, so that comparison with alkalinity values are possible.

Results and discussion

Results of the chemical and isotopic analyses are given in Table 1. The $\delta^{13}C$ values of DIC vary from -21.5‰ to -7.9‰. The $\delta^{13}C$ of DIC in carbonate-rich groundwater, such as in SW Michigan, is controlled by the $\delta^{13}C$ of soil carbon dioxide and native carbonate minerals. The $\delta^{13}C$ of soil carbon dioxide reflects that of the vegetation in the given area and may be modified by processes such as diffusion of carbon dioxide from the vadose zone (Cerling, 1984). Carbon isotope values for the vegetation are expected to average about -26‰ or -13‰ for C_3 or C_4 type vegetation, respectively (Deines, 1980). The organic carbon fraction in soils is a reasonable proxy for the vegetation (Krishnamurthy and Bhattacharya, 1989). The $\delta^{13}C$ values of organic carbon from several soil samples and tissues of corn, the major agricultural crop at the study site are given in Table 1. The mean isotope value of -24.3 ± 1.4‰, indicates contribution from a predominantly C_3 type vegetation to the soil organic carbon. Soil carbonates from the study region gave an average $\delta^{13}C$ value of +1.5 ± 1‰, which is within the range reported for parent carbonates in the Michigan basin (Taylor and Sibley, 1986; Middleton et al., 1990). The equilibrium $\delta^{13}C$ of DIC is expected to be around -12‰ based on the measured isotope ratios of soil organic carbon (-24.3‰) and carbonates (+1.5‰). If diffusion to the atmosphere is important, the $\delta^{13}C$ of soil CO_2

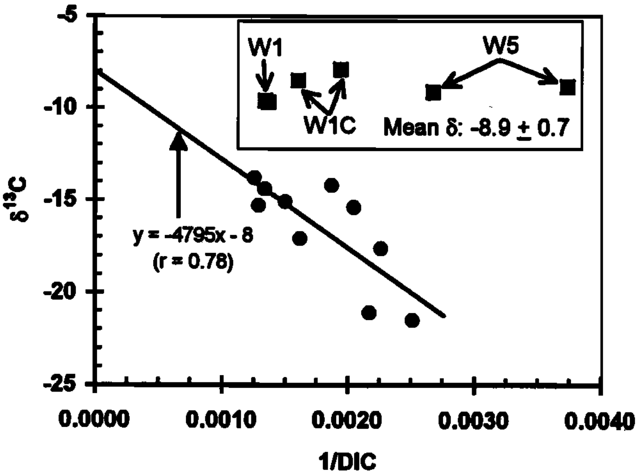

Figure 2. Relationship between "1/DIC" and $\delta^{13}C$ of DIC. The regression line excludes the outlier enclosed by the box.

would be expected to be around -20‰ and the $\delta^{13}C$ of DIC would be around -9‰ (Cerling, 1984).

The $\delta^{13}C_{DIC}$ vs. DIC^{-1} plot is useful in interpreting $\delta^{13}C$-DIC relationships (Grossman et al., 1989). The y-intercept of this plot gives the $\delta^{13}C$ value of the starting component. Such a plot shows that the samples fall into two distinct fields (Fig. 2). One of them comprising samples W1, W1C and W5 have a relatively narrow range of $\delta^{13}C$ values of -8.9‰ but a rather wide range of DIC concentrations. The other group falls on well correlated line with a y-intercept of -8‰. Interestingly, the former three samples are also the ones with the highest nitrate concentrations. As described previously, a $\delta^{13}C$ value of -8‰ is reasonable for background DIC where soil CO_2 modified by diffusion dissolves parent limestone. The significance of the linear $\delta^{13}C_{DIC}$ vs DIC^{-1} relationship with a negative slope for the second set of samples is that they fall on a mixing line with one end member being background DIC ($\delta^{13}C$ of -8‰) and another with a lower value.

An important observation that can be made from the data in Table 1 is that all the water samples show DIC concentrations in excess of alkalinity. DIC measured here represents the total carbon released by phosphoric acid i.e. HCO_3^- + CO_2 and CO_3^{2-}. CO_3^{2-} is negligible at the pH conditions measured for the samples. On the other hand, alkalinity is predominantly carbon in the bicarbonate. Excess DIC in these groundwaters must be present as undissociated CO_2 which is why the procedure of acid titration to determine alkalinity does not detect it (Hem, 1974). This "excess" CO_2 over alkalinity can be expressed as a "per cent excess" by:

$$ex_{CO_2} = (DIC - Alkalinity / Alkalinity) \times 100 \quad ......(1)$$

These values are also given in Table 1.

Several reactions consume organic carbon to produce CO_2 such as microbial reactions utilizing O_2, Fe, Mn and SO_4 and methanogenesis. When sufficient carbon and nitrates are available, denitrification involving oxidation of carbon will be the most preferred pathway (Herczeg et al., 1991). Given the substantial use of nitrate fertilizers at this site and the suboxic to anoxic conditions below the water table, a process that could introduce carbon with low $\delta^{13}C$ values is denitrification (Smith et al., 1991; Bohlke and Denver, 1995). This hypothesis is further supported by the low dissolved oxygen levels in samples with the lowest nitrate levels (Table 1). Therefore, we argue that the other processes of organic carbon consumption mentioned above are less likely.

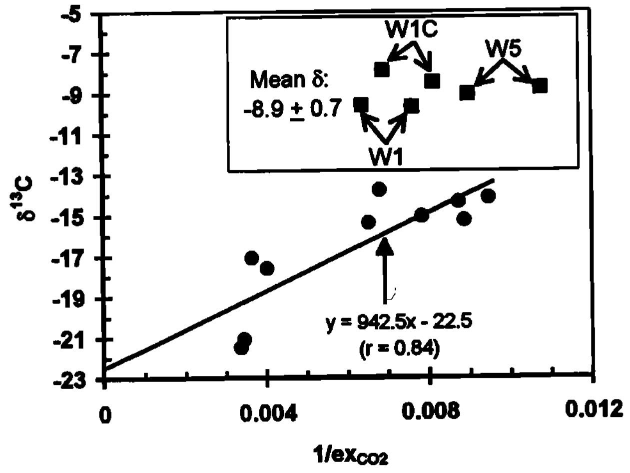

Figure 3. Relationship between "$1/ex_{CO_2}$" and $\delta^{13}C$ of DIC. The regression line excludes the outlier enclosed by the box.

Denitrification occurs in sub-oxic and anoxic environments where certain strains of bacteria use readily available organic carbon and nitrates (oxidants) and convert nitrates into molecular nitrogen and generate carbon dioxide gas according to the following simplified reaction:

$$5C_{org} + 4NO_3^- + 4H^+ = 2N_2 + 5CO_2 + 2H_2O$$

This reaction provides a means by which CO_2 in excess of what is normally present as undissociated CO_2 in a CO_2-H_2O system (i.e., H_2CO_3 + HCO_3^-) can be introduced in these groundwaters. Also, since the carbon for denitrification derives from an organic source which is isotopically more ^{13}C depleted compared to that of the carbonate/bicarbonate pools (i.e., alkalinity), the $\delta^{13}C$ values of the DIC containing this carbon will be more negative than the DIC which either does not contain this carbon or the DIC whose $\delta^{13}C$ has been modified significantly by carbonate dissolution. Thus combined DIC-alkalinity measurements along with the carbon isotope ratios of DIC can be a powerful technique in the investigation of denitrification in nature. For this, one can again make use of a plot of the type shown in Fig. 3, where the $\delta^{13}C_{DIC}$ is plotted against the reciprocal of "excess CO_2" ($1/ex_{CO_2}$). The y-intercept of this line would give the $\delta^{13}C$ value of the carbon used for denitrification. As shown in Fig. 3, the samples with lower nitrate levels define a well-correlated line with a y-intercept of -22.5‰. This intercept is well within the range of $\delta^{13}C$ values of organic carbon obtained for the aquifer materials and is indeed a reasonable value for the organic substrate utilized by denitrifying bacteria. Assuming therefore that this line represents a mixing line between two DIC pools with end member $\delta^{13}C$ values of -8‰ (carbonate dissolution) and -22.5‰ (denitrification), one can use the measured isotope ratios and a simple mass balance equation to calculate the fraction of carbon derived from denitrification added to the DIC pool of each of these samples. These calculated values are given in Table 1. It is noteworthy that the highest degree of such contribution has taken place in water from wells W3, W4 and W6. This can be attributed to the fact that these waters are close to depressions where accumulation of organic matter provides a significant source of carbon substrate (see Fig. 1). Spatial variability in the observed denitrification is probably dictated by the availability of organic carbon substrate and seems to be localized at this site. It is also noteworthy, based on the $\delta^{13}C$ values, that the carbon substrate does not seem to have been derived from corn, although it is a major agricultural crop in this region. This mixing model does not take into account groundwater flow conditions. Moreover, the spatial distribution of the wells and the vertical sampling intervals are not extensive enough to monitor changes in DIC induced by groundwater evolution.

The DIC-$\delta^{13}C$ distribution of the three samples W1, W1C and W5 (Figs. 2 & 3) reveals that their $\delta^{13}C$ values have a rather narrow range, but the ex_{CO_2} shows a larger spread. Two likely explanations are: (1) the DIC in these waters formed under conditions where a large reservior of CO_2 is available for carbonate dissolution. Support for this this hypothesis is indicated by the relatively elevated levels of calcium and magnesium in these samples, (2) this large reservior of CO_2 represents CO_2 derived from corn plants ($\delta^{13}C$ = -12‰) that has been modified by diffusion. The importance of determining the $\delta^{13}C$ along with DIC and alkalinity is thus clearly underscored. That is to say, in following the process of denitrification, the parameter "ex_{CO_2}" as defined above, and the $\delta^{13}C$ values in combination determine if denitrification is taking place. Our observation that substantially high levels of undissociated CO_2 exist in most of these waters has an important implication: measurements of alkalinity alone without measurement of total DIC in these type of waters is of

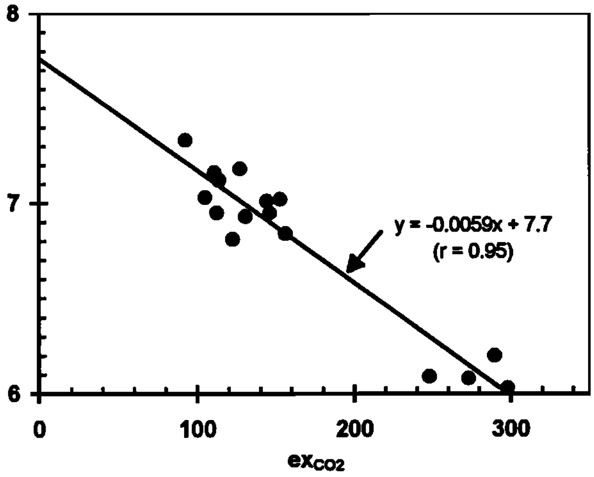

Figure 4. Relationship between pH and "ex_{CO2}".

limited value. This is evident from Fig. 4, which shows that the pH of these waters is determined by the undissociated carbon dioxide. Therefore the assumption that alkalinity represents total inorganic carbon species in these environments may not be valid. Determination of DIC concentration is a better approach than calculating it based on pH and alkalinity imposing equilibrium conditions.

Conclusions

Studies to monitor denitrification normally make use of the stable isotope ratios of nitrogen and sulfur, the choice mostly being the isotopes of nitrogen. This is based on simple mixing models where the end members are the isotope ratios of the added fertilizers and that of the nitrate species in groundwater. The combination of DIC, alkalinity, pH and $\delta^{13}C$ measurements proposed here makes use of one of the chief by-products of denitrification, namely carbon dioxide, and appears to be a simple and rapid method that can be used in conjunction with other techniques to monitor denitrification. Investigations carried out in a nitrate impacted site in southwestern Michigan verified this approach. Groundwater with lower nitrate levels and dissolved oxygen had higher amounts of undissociated CO_2 and lower $\delta^{13}C$ values. The occurrence of excess CO_2 suggested that in environments similar to these, DIC measurments provide a more accurate description of the total inorganic carbon in the system.

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