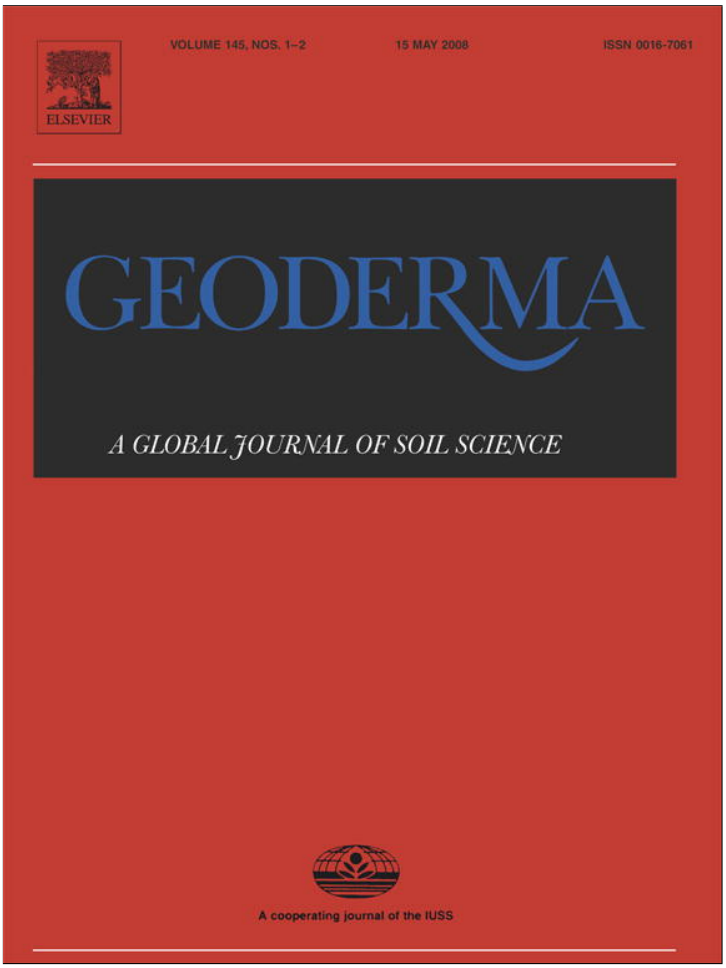

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Carbon and nitrogen stable isotope ratios can estimate anionic polyacrylamide degradation in soil

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Abstract

Water-soluble anionic polyacrylamide (PAM) is applied to millions of hectares worldwide as a highly effective erosion-preventing and infiltration-enhancing polymer, when applied at rates of 1 to 2 kg ha^{-1} (i.e., 1 to 10 g m^{-3}) in furrow water. PAM degradation has not directly been measured in soil. We tested the ability of stable isotopes of C and N at natural abundance to estimate PAM degradation rates. Values of $\delta^{13}C$ were related to anionic PAM concentration in a positive curvilinear relationship in a low-C, low-N, Durinodic Xeric Haplocalcid (Portneuf series) soil. The other soils with higher organic C or N concentrations did not show significant relationships between PAM concentrations and $\delta^{13}C$ values. The $\delta^{15}N$ values were not related to anionic PAM concentration in any of the soils tested. When 2691 active ingredient (ai) kg PAM ha^{-1} was applied to the Durinodic Xeric Haplocalcid soil, 49% and 74% of the PAM was degraded after 6 and 12 yr respectively. When 5382 kg ai PAM ha^{-1} was applied to the Durinodic Xeric Haplocalcid soil, 13% was degraded after 6 yr, and 73% of the PAM was degraded after 12 yr. We calculated PAM degradation rate based on $\delta^{13}C$for the Durinodic Xeric Haplocalcid soil to be 9.8% yr^{-1}. Further testing using labeled PAM is necessary to estimate degradation rates in higher C soils, and to determine what portion of the C is released from decomposing PAM is emitted to the atmosphere, incorporated into soil organic matter and living microbial biomass.

Keywords: Polyacrylamide degradation; Stable isotopes; Soil type; C and N content

1. Introduction

Water-soluble anionic polyacrylamide (PAM) is a highly effective erosion-preventing and infiltration-enhancing polymer, when applied at rates of 1 to 10 g m^{-3} in furrow irrigation water (Lentz et al., 1992; Lentz and Sojka, 1994; McCutchan et al., 1994; Trout et al., 1995; Sojka and Lentz, 1997; Sojka et al., 1998a,b). Coulombic and Van der Waals forces attract soil particles to PAM (Orts et al., 1999, 2000). These surface attractions stabilize soil structure by enhancing particle cohesion, thus increasing resistance to shear-induced detachment and preventing transport in runoff. The few particles that detach are quickly flocculated by PAM, settling them out of the transport stream. Modest amounts (200–600 mg Ca kg^{-1} soil) of Ca^{+} in the water shrink the electrical double layer surrounding soil particles and bridge the anionic surfaces of soil particles and PAM molecules, enabling flocculation (Orts et al., 2001; Wallace and Wallace, 1996).

PAM delivery via irrigation water is very efficient, because it needs only to stabilize the thin veneer of soil directly active in the erosion process. In furrow irrigation, PAM treats only about 25% of the field surface area to a few millimeters depth, thus only 1–2 kg ha^{-1} of PAM per irrigation is required to halt erosion and improve infiltration. PAM achieves its result by stabilizing soil surface structure and pore continuity. PAMs were first sold for erosion control in the U.S. in 1995, and by 1999 about 400,000 ha were PAM-treated in the U.S. The U.S. market is expected to continue to grow as water quality improvements are mandated by new federal legislation and court action, and since PAM use is one of the most effective,

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economical and least intrusive management approaches recently identified that can meet the needed water quality improvement (Sojka et al., 2007). PAM reduced sediment in furrow irrigation runoff by 94% in 3 yr of studies in Idaho (Lentz and Sojka, 1994). The original 1995 National Resource Conservation Service PAM application method called for dissolving 10 g m^{-3} PAM in furrow inflow water as it first crosses a field (i.e., water advance, which is, typically the first 10 to 25% of an irrigation duration). Using this method, PAM dosing is halted when runoff begins. The PAM applied during water advance generally prevents erosion throughout a 24 h irrigation. Application amounts dissolved in the advancing stream usually total 1–2 kg ha^{-1} per irrigation. For freshly formed furrows, Lentz et al. (2000) reported that on 1–2% slopes, the effectiveness of applying PAM at a uniformly dosed inflow concentration varied with inflow rate, PAM concentration, duration of furrow exposure, and total amount of PAM applied. Erosion control with PAM was similar for three application methods: 1) application of 10 kg ML^{-1} dissolved in the advance, 2) application of 5 kg ML^{-1} during advance, followed by 5 to 10 min of 5 kg ML^{-1} re-application every few hours, or 3) continuous application of 1 to 2 kg ML^{-1} for the entire irrigation period. Continuous application of 0.25 kg ML^{-1} controlled erosion about one third less effectively.

PAM degradation occurs slowly in soils as a result of chemical, photochemical, biological and mechanical processes (i.e., such as abrasion, freezing, thawing) because of the molecule's enormous size. Abiotic processes break the molecule into progressively shorter units over time. When polymer units are 6–7 monomer units long they are degraded by soil microorganisms (Hayashi et al., 1993). PAM degradation has been indirectly estimated to degrade in soil at approximately 10% yr^{-1} (Azzam et al., 1983). PAM degradation in soil has been measured only indirectly because the substance adheres to soil particles so tightly that it can not be adequately extracted so that it may be analyzed by gas or high performance liquid chromatographic techniques. Several new techniques for removal of PAM adsorbed to soil and for PAM analysis have recently been explored (Lu and Wu, 2003a,b, 2002, 2001; Lu et al., 2003, 2002). Although PAM can be removed from soil with vigorous chemical stripping, questions remain concerning the effectiveness of PAM removal and the accuracy of determination of the analyte. The strong chemical reactions necessary to extract PAM from soil often change its molecular conformation, undermining the quantification (Sojka et al., 2007). The pathway for PAM degradation has been described when PAM was exposed to 95 °C, fluorescent light and UV radiation (Caulfield et al., 2003; Stahl et al., 2000; Diffy, 1991; Suzuki, 1978; 1979). The existing information on degradation rates of PAM in soil and water is insufficient to accurately predict the residence time of the compound in various ecosystems. This lack of reliable information has repeatedly subjected PAM users to safety questions from environmental watch groups and government regulators. This has been one of the most persistent and difficult obstacles in most situations to garnering full public sector endorsement of PAM technology for environmental protection.

Stable isotope ratios of organisms reflect the source ratios, with adjustment for discrimination during assimilation, of the molecules they assimilate. (DeNiro and Epstein, 1978, 1981). Stable isotope ratios have been used to determine degradation of a variety of organic compounds in soil (Somsamak et al., 2006; Smernik, 2005; Meckenstock et al., 2004; Boschker and Middelburg, 2002).

Biodegradation isotope effects are typically limited to small molecules that can readily permeate cell membranes of heterotrophic organisms (Philp, 2007) Biodegradation of long chain *n*-alkanes and multi-ring polyaromatic hydrocarbons do not result in measurable carbon isotopic fractionation (Philp, 2007; Sun et al., 2005). Since anionic PAM is not an alkane, the compound's degradation by soil microorganisms can not be directly compared to long chain *n*-alkanes. However, anionic PAM is a long chain linear molecule with a molecular weight of 12 to 15 Mg mol^{-1} and it is likely that anionic PAM degradation should not result in significant $\delta^{13}C$ or $\delta^{15}N$ fractionation.

The stable isotope ratios of light elements (e.g., C, N, O, H and S) are measured on isotope ratio mass spectrometers with fixed magnets and Faraday cups positioned to detect a small number of masses (Kreuzer-Martin, 2007). The configuration generates high sensitivity and precision, enabling accurate measurement of isotope ratios. Isotope ratios can also be determined by quadrupole mass spectrometers that require a higher concentration of the rare isotope than isotope ratio mass spectrometers (IRMS) (Kreuzer-Martin, 2007; Meckenstock et al., 2004). IRMS measure isotope ratios in comparison to reference materials, which are analyzed in parallel with the samples. In natural abundance studies, the isotope ratios are expressed in relation to international standards as delta values (δ) in parts per thousand (‰) (Kreuzer-Martin, 2007; Meckenstock et al., 2004). Combustion is used for carbon analysis as CO_2 and nitrogen as N_2.

PAM degradation has not directly been measured in soil or water. The objective of our research was to determine the efficacy of natural abundance of the carbon ($^{13}C/^{12}C$) and nitrogen stable isotope ratios ($^{15}N/^{14}N$) to measure the concentration of anionic PAM in four different soils and to measure PAM degradation rates.

2. Materials and methods

2.1. Soils

The Portneuf soil (coarse-silty, mixed, super active, mesic Durinodic Xeric Haplocalcid) was sampled from the 0–5 cm of an agricultural field in Kimberly, Idaho. The silt loam soil contains 10–21 g kg^{-1} clay and 60–75 g kg^{-1}silt, and organic matter of approximately 13 g kg^{-1}. Saturated paste extract electrical conductivity (EC) of these calcareous soils range from 0.7 to 1.3 dS m^{-1}, with exchangeable sodium percentage (ESP) of 1.4 to 1.7, and pH of 7.6–8.0, and a $CaCO_3$ equivalent of 2–8%. The soil contained 1.50 g C kg^{-1} soil , 0.09 g N kg^{-1} soil and the C:N was 22.0. Soil nutrient and microbial characteristics are described in (Entry and Sojka, 2003; Sojka et al., 2006).

The Bluepoint–Kokan soil (pale brown fine sandy Typic Torripsamment) was sampled from the 0–5 cm of an agricultural field near Sandoval County, New Mexico. The soil formed in a sandy alluvium. Vegetation on the soil was short grasses and broom snakeweed. Mean annual precipitation varies from 16–25 cm per year. The soil has 10–21 g kg^{-1} clay and 60–75 g kg^{-1}silt. The soil contained 0.13 g C kg^{-1} soil, 3.90 g N kg^{-1} soil and the C:N was 0.03. Saturated paste extract electrical conductivity (EC) of these basic soils range from 0.7 to 1.0 dS m^{-1}, with exchangeable sodium percentage (ESP) of 1.0 to 147 and pH of 7.6–7.8.

The Latachco soil (fine-silty, mixed, superactive, frigid Argiaquic Xeric Argialbolls) was sampled from the 0–5 cm of an agricultural field near Pullman, Washington. The Latahco series consists of very deep loess soils that formed in alluvium from the surrounding uplands. The soil is approximately 20 g kg^{-1}sand, 20 g kg^{-1} clay and 60 g kg^{-1} silt with a pH of 5.9 (Bae and Knudsen, 2001). The soil contained 19.3 g C kg^{-1}, 1.44 g N kg^{-1} and the C:N was 13.4. The average annual precipitation is about 45 cm and the average annual temperature is 17 °C.

The Norfolk sandy loam (fine-loamy, siliceous, thermic Typic Kandiudult) soil was sampled from the 0–5 cm (Ap horizon) from a long-term research site that had conventional (disked) and conservation (non-disked) tillage plots established in 1978 near Florence, South Carolina. The thermic Typic Kandiudult soil was a sandy loam that formed in coastal marine sediments. It has an Ap horizon that had been tilled over the years to a depth of about 20 cm. The Ap horizon contained 9.50 kg C kg^{-1}, 0.70 kg N kg^{-1} and the C:N was 12.6. Below the tilled layer, it had an eluviated E horizon that typically varies in depth from 30 to 100 cm and when re-compacted developed penetration resistance that restricts root growth. Both the Ap and E horizons had less than 5 to 6 g kg^{-1} soil organic matter, 1 to 4 cmol kg^{-1} cation exchange capacity, and 20 to 80 g kg^{-1} clay content. The Ap and E horizons overlaid a sandy clay loam Bt horizon that extended beyond 60 cm depth. The Bt horizon typically had less than 5 g kg^{-1} organic matter, 2 to 5 cmol kg^{-1} cation exchange capacity, and 200 to 400 g kg^{-1} clay content. The field was planted with corn (*Zea mays* L.) from 2002 through 2005 years and fertilized with 110 N kg ha^{-1} as NH_4NO_3.

2.2. Polyacrylamide concentrations and stable isotope ratios

Soil samples were taken from four different soils types described above. Anionic PAM was added to each soil at 0, 10, 100, 1000 and 10,000 mg ai PAM kg^{-1} soil (Table 1). There were four soils each given the above five PAM concentrations.

Table 1
PAM concentrations applied to four soils in replicate treatments

mg ai PAM kg^{-1} soil	G ai PAM	Treatment
0	0.000000	No PAM, 100 ml water only (control)
10	0.000055	PAM dissolved in 100 ml water
100	0.00055	PAM dissolved in 100 ml water
1000	0.0055	PAM dissolved in 100 ml water
10000	0.055	PAM dissolved in 100 ml water

The experiment contained three replicates for a total of 60 samples, which were analyzed by two separate laboratories.

2.3. Sampling procedures and PAM application

Soil samples were collected from the top 5.0 cm of mineral soil in three separate 1-m^2 areas of each soil type. 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. We air-dried the soils to a water content of 25 g kg^{-1} by placing the soil in a drying oven at 70°C for 48 h.

We mixed PAM in 100 ml reverse osmosis water and added the mixture to 100 g soil (Table 1). One hundred g of each soil was placed in a 150 ml French square bottle and water containing the above stated amounts of PAM and soil was added. Soil and PAM was thoroughly mixed, placing the bottles on a shaker for 30 min. Water content of soils were adjusted to 25 g kg^{-1} by placing the soil in a drying oven at 70^{N}C for 48 h and mixed by placing them in a shaker for 30 min. A 2-mg subsample was randomly taken from each soil — PAM sample was analyzed for $\delta^{13}C$ and $\delta^{15}N$ for sand, silt, and clay content, electrical conductivity, and exchangeable sodium percentage.

2.4. Stable isotope ratio analysis

For isotope ratio analysis, 2 mg±10% soil samples were weighed and placed into tin capsules. Carbon and nitrogen isotope ratios of each sample were determined on a Finnigan-MAT Delta S isotope ratio mass spectrometer (IRMS, Bremen, Germany) interfaced with an Elemental Analyzer (Model 1108; Carlo Erba, Milan Italy). We split each soil sample from the laboratory study and sent identical samples to both the Stable Isotope Ratio Facility for Environmental Research (SIRFER) in the Biology Department at the University of Utah and the Idaho Stable Isotopes Laboratory at the University of Idaho in the Forest Resources Department for $\delta^{13}C$ and $\delta^{15}N$ analysis. Both the SIRFER laboratory and the Idaho Stable Isotopes Laboratory each calibrate their own internal laboratory standards relative to the international standards and include multiple samples of the internal standard in each set of unknown samples to be analyzed. Instrument precision, based on repeated measurements of these internal laboratory standards, was 0.2‰ for both carbon and nitrogen isotope ratios. Mean $\delta^{13}C$ for anionic polyacrylamide was −29.3‰ and mean $\delta^{15}N$ was 0.2‰ (N=5). Anionic PAM (–$CH_2CHCONH_2$–) is composed of 51% C and 20% N by weight as there are 3 C atoms for each N atom in the molecule.

Stable isotope ratios were measured relative to internationally recognized standards. The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as relative difference per mil (l) using the equation:

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000,$$

where X=^{13}C or ^{15}N, and R=$^{13}C/^{12}C$ or $^{15}N/^{14}N$. The ratio of ^{13}C to ^{12}C was compared to the Peedee belemnite standard, for which

Table 2
Carbon and nitrogen content and stable isotope ratios of the four study soils

A. Nitrogen and carbon contents

Soil	N	C content (%)		N content (%)		C/N	
		Mean	95% CI	Mean	95% CI	Mean	95% CI
Typic Torripsamment	15	0.013	0.002	0.390	0.017	0.03	0.01
Durinodic Haplocalcid	15	0.150	0.009	0.009	0.003	22.0	6.50
Typic Kandiudult	15	0.950	0.462	0.070	0.030	12.6	1.45
Argiaquic Argialbolls	15	1.933	0.024	0.144	0.002	13.4	0.22

B. Stable isotope ratios

Soil	N	δ^{13}C (‰)		δ^{15}N (‰)	
		Mean	95% CI	Mean	95% CI
Typic Torripsamment	15	−9.3	0.16	−3.6	1.09
Durinodic Haplocalcid	15	−12.9	0.81	−1.6	0.86
Typic Kandiudult	15	−22.7	1.12	−3.6	0.55
Argiaquic Argialbolls	15	−25.5	0.05	−7.4	0.18

$R_{standard}$ = 1.1237 atom % ^{13}C (Craig, 1957; Coplen, 1996). For ^{15}N/^{14}N, N_2 in air was used as the standard, and $R_{standard}$ = 0.3663 atom % ^{15}N (Mariotti, 1983).

2.5. Laboratory study statistical analyses

All dependent variables were tested for normal distribution. Residuals were equally distributed with constant variances. Regressions were determined with PAM concentration as the independent value and δ^{13}C and δ^{15}N as independent values with Statistical Analysis Systems (SAS, 1996).

2.6. Polyacrylamide degradation rates in the field

The PAM degradation study was conducted on an experiment station site that had large accumulative additions of PAM over 3–6 yr periods. There was little if any effect on microbial diversity and effects on soil microbial biomass, though measurable, were inconsistent and small considering the massive amounts of PAM added (Sojka et al., 2006). We used the δ^{13}C to determine the amount of anionic PAM remaining in the Xeric Haplocalcid (Kimberly) soil because we obtained the best correlation with the amount of anionic PAM in soil with the δ^{13}C in that soil, the site had large quantities of the chemical applied over a six year period, and the soil microbiological community had been characterized.

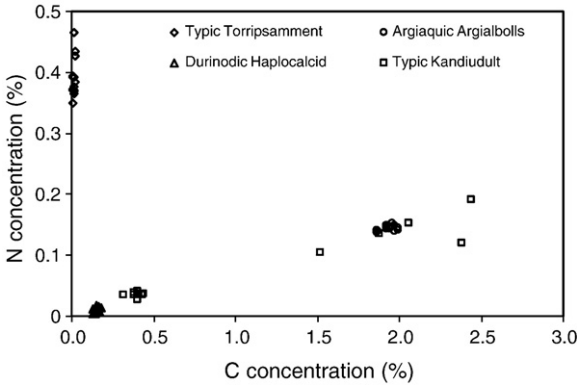

Fig. 1. Nitrogen and carbon contents of the four study soils.

2.7. Field study site and experimental design

The study was conducted at the USDA Agricultural Research Service's Northwest Irrigation and Soils Research Laboratory in Kimberly, Idaho. The soil in the test area was Portneuf silt loam (coarse-silty, mixed, superactive mesic Durinodic Xeric Haplocalcid) described above. Slope on this site was approximately 1.5%. Soil physical and chemical parameters on these treatments are described in detail in Sojka et al. (2006).

The experimental design was a randomized complete block with three replications. Treatments were: 5382 kg active ingredient (ai) PAM ha^1, 2691 kg ai PAM ha^1 and a control (no PAM applied) with 3 replications (plots) of each treatment. Plots were treated 5 yr prior to sampling. We took three samples in June, July and August, 2000 and 2006 from each plot. Plots were 40 m long × 4 m wide. We took a total of 54 samples (3 treatments × 3 replicated plots × 3 separate samples taken within each plot × 2 sampling years).

2.8. Polyacrylamide application

The polyacrylamide copolymer used was a dry granular material having an approximate molecular weight of 12–15 Mg mol^{-1}, with an 18% negative charge density (Sojka et al., 2007; Barvenik, 1994). PAM application involved the spread of granular PAM on the surface of an approximately 0.1 m^2 area in the furrow, corresponding to the first meter of furrow below inflow spigots (Sojka et al., 1998c). We applied 897 kg ai PAM ha^{-1} in the spring every year for 3 yr to plots receiving 2691 kg PAM ha^{-1}. We applied 897 kg PAM ha^{-1} in the spring every year for 6 yr to plots receiving 5382 kg PAM ha^{-1}. Since the PAM contains 51% C and 20% N, PAM treatments received 457 kg C ha^{-1} and 179 kg N ha^{-1} each year while receiving 897 kg ha^{-1} PAM. Fertilizer, pesticide and water application are

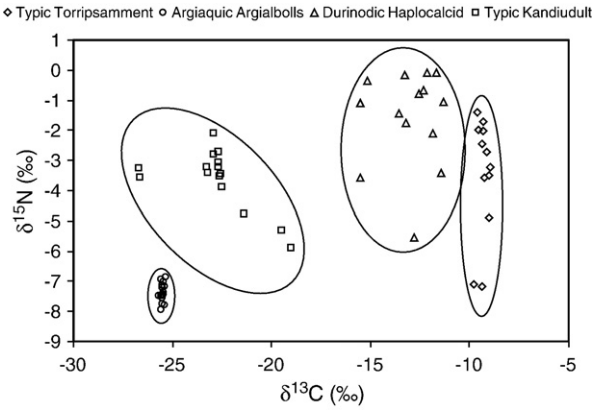

Fig. 2. Dual isotope plots of the four study soils after addition of PAM. See text for PAM concentrations.

Table 3
Pearson correlations among PAM concentration, carbon and nitrogen content, and stable isotope ratios for four soils with applied PAM ($N=60$)

	Log_{10} [PAM]+1	Percent carbon	Percent nitrogen	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Log_{10}[PAM]+ 1	1				
Percent carbon	−0.127	1			
Percent nitrogen	0.239	−0.066	1		
$\delta^{13}C$ (‰)	0.152	***−0.825***	***0.368***	1	
$\delta^{15}N$ (‰)	0.079	***−0.737***	−0.230	***0.604***	1

Values in bold and italics are significant ($P \geq 0.012$) with Bonferroni adjusted probabilities.

described in Sojka et al. (2006). Following application of PAM each year soil was roto-tilled to a depth of 15 cm to incorporate the freshly applied PAM.

2.9. Sampling and analysis

Three separate 2.5-cm diameter soil cores were collected from the top 30 cm of mineral soil from the above different locations in each plot in June, July and August, 2000 and 2006. Samples of mineral soil collected from each plot were as described above. Stable isotope ratios for soils taken from field plots were analyzed as described above.

All dependent variables were tested for normal distribution. Data were then analyzed by means of analysis of variance procedures (ANOVA) for a split plot design with Statistical Analysis Systems (SAS, 1996). Residuals were equally distributed with constant variances. Differences reported throughout are significant at $P \leq 0.05$, as determined by the protected Least Squares Means (LSM) test (Kirk, 1982).

3. Results

3.1. Polyacrylamide concentrations and stable isotope ratios

We split each soil sample from the laboratory study and sent identical samples to both the Stable Isotope Ratio Facility for Environmental Research (SIRFER) in the Biology Department at the University of Utah and the Idaho Stable Isotopes Laboratory at the University of Idaho in the Forest Resources Department for $\delta^{13}C$ and $\delta^{15}N$ analysis. The data from each laboratory agreed with the other extremely well (data not shown). Data reported for PAM degradation was analyzed by the SIRFER laboratory only. All results reported here are from the SIRFER laboratory. Carbon and N content of the soils before PAM application varied widely (Table 2A, Fig. 1). The Typic Torripsamment (Bluepoint–Kokan) soil had the lowest carbon content, but the highest nitrogen content. The Durinodic Xeric Haplocalcid (Portneuf) soil had the lowest nitrogen content. The lowest $\delta^{13}C$ and $\delta^{15}N$ values occurred in the Argiaquic Xeric Agiaboll (Latachco) soil (Table 2B, Fig. 2). Over all four soils, $\delta^{13}C$ and $\delta^{15}N$ were both negatively correlated with C content (Table 3; $P<0.001$). The discriminant function based on C and N concentrations and $\delta^{15}N$ and $\delta^{13}C$ values correctly classified all four soils with at least 93% accuracy (Table 4A). Using the first two unstandardised canonical discriminant functions (Table 4B), the soils were grouped better than with C and N or $\delta^{13}C$ and $\delta^{15}N$ alone (Figs. 1 and 2 compared to Fig. 3). Increases in canonical discriminant function 1 were related to decreasing percent N and $\delta^{13}C$, whereas increases in canonical

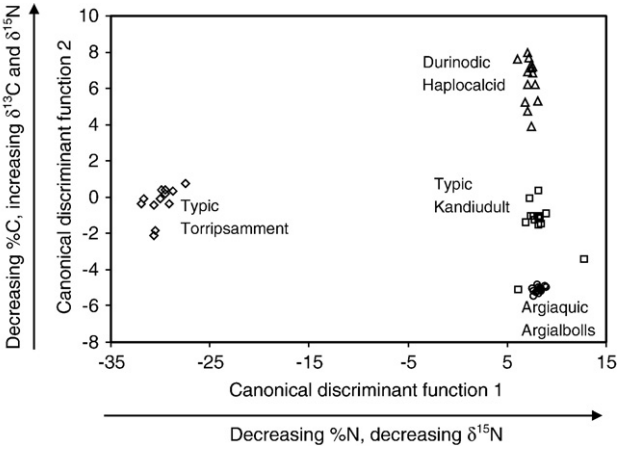

Fig. 3. The first two unstandardised canonical discriminant functions for four soils based on C and N concentration and $\delta^{13}C$ and $\delta^{15}N$ values.

Table 4
Discriminant function analysis of four soils based on C and N concentration and $\delta^{15}N$ values

A. Classification matrix

Soil	Typic Kandiudult	Durinodic Haplocalcid	Typic Torripsamment	Argiaquic Argialbolls	Percent correct
Typic Kandiudult	14	0	0	1	93
Durinodic Haplocalcid	0	15	0	0	100
Typic Torripsamment	0	0	12	0	100
Argiaquic Argialbolls	0	0	0	15	100
Total	14	15	12	16	98

B. Unstandardised canonical discriminant functions (CDF)

	CDF1	CDF2	CDF3
Constant	4.704	16.125	3.893
Percent C	6.134	1.224	1.537
Percent N	−98.155	−23.204	−6.457
$\delta^{13}C$	−0.174	0.697	0.345
$\delta^{15}N$	−0.203	0.326	−0.497

Table 5
Anionic polyacrylamide (AN 923 SH) concentration in mg active ingredient PAM kg^{-1} soil relative to ^{13}C and ^{15}N content expressed as $\delta^{13}C$ or $\delta^{15}N$ in ‰

Soil	$\delta^{13}C$		$\delta^{15}N$	
	r^2	Regression equation	r^2	Regression equation
Durinodic Haplocalcid	0.943	$Log_{10}PAM+1=-38.94-(5.331*\delta^{13}C)-(0.165*\delta^{13}C^2)$	0.001	$Log_{10}PAM+1=2.017-(0.033*\delta^{15}N)+(0.000*\delta^{15}N^2)$
Typic Torripsamment	0.220	$Log_{10}PAM+1=-151.4-(36.10*\delta^{13}C)-(2.095*\delta^{13}C^2)$	0.073	$Log_{10}PAM+1=0.970-(1.189*\delta^{15}N)+(0.135*\delta^{15}N^2)$
Typic Kandiudult	0.001	$Log_{10}PAM+1=-0.739-(0.236*\delta^{13}C)-(0.005*\delta^{13}C^2)$	0.187	$Log_{10}PAM+1=10.335+(4.035*\delta^{15}N)+(0.447*\delta^{15}N^2)$
Argiaquic Argialbolls	0.335	$Log_{10}PAM+1=13110+(1019*\delta^{13}C)+(19.81*\delta^{13}C^2)$	0.181	$Log_{10}PAM+1=186.8+(48.49*\delta^{15}N)+(3.172*\delta^{15}N^2)$

discriminant function 2 were related to decreasing percent C and increasing $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3).

In experiment 1, values of $\delta^{13}C$ were related to anionic PAM concentration in a positive curvilinear relationship in a low-C, low-N, Durinodic Xeric Haplocalcid (Portneuf) soil from Kimberly, Idaho (Table 5, Fig. 4). Soils with higher C or N concentrations did not show significant relationships between PAM concentrations and $\delta^{13}C$ values (Typic Torripsamment from Albuquerque, New Mexico, Typic Kandiudult from Florence, South Carolina, and Xeric Argialboll from Pullman, Washington); $r^2 \leq 0.335$, $P \geq 0.087$). Values of $\delta^{15}N$ were not related to anionic PAM concentration in any of the soils tested ($r^2 \leq 0.187$, $P \geq 0.289$). PAM concentrations in soils as measured by $\delta^{13}C$ from the SIRFER laboratory correlated with PAM concentrations in soils as measured by the Idaho Stable Isotopes Laboratory (Xeric Haplocalcid $r^2=0.85$; Typic Kandiudult $r^2=0.48$; Typic Torripsamment $r^2=0.96$; Xeric Argialboll $r^2=0.97$). PAM concentrations in soils as measured by $\delta^{15}N$ from the SIRFER laboratory were not well correlated with PAM concentrations in soils as measured by the Idaho Stable Isotopes Laboratory (Xeric Haplocalcid $r^2=0.14$; Typic Kandiudult $r^2=0.34$; Typic Torripsamment $r^2=0.34$; Xeric Argialboll $r^2=0.24$).

3.2. Polyacrylamide degradation rates in the field

In experiment 2, we applied 897 kg ai PAM ha^{-1} in the spring annually for 3 yr to plots of the Durinodic Xeric Haplocalcid (Portneuf) soil from Kimberly, Idaho, that received 2691 kg PAM and 897 kg ai PAM ha^{-1} in the spring annually for 6 yr to plots receiving 5382 kg PAM ha^{-1}. When 2691 kg PAM ha^{-1} was applied to the soil, we measured 1317 kg ai PAM ha^{-1} in 2000, indicating 49% was degraded after 6 yr and in 2006 we measured 691 kg ai PAM ha^{-1}, indicating that 74% of the PAM was degraded after 12 yr (Table 6). When 5382 kg ai PAM ha^{-1} was applied to the soil from 1995 to 2000, we measured 4675 kg ai PAM ha^{-1} in 2000 indicating 13% was degraded after 6 yr and in 2006 we measured 1466 kg ai PAM ha^{-1} indicating 73% of the PAM was degraded after 12 yr (Table 6). We calculated PAM degradation rates based on $\delta^{13}C$ to be 9.8% yr^{-1}.

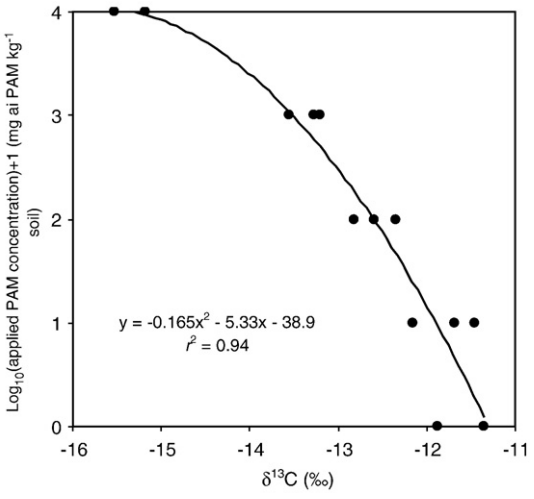

Fig. 4. Relationship of $\delta^{13}C$ to applied PAM concentration for a low-carbon Durinodic Haplocalcid soil in Kimberly, Idaho.

4. Discussion

4.1. Polyacrylamide concentrations and stable isotope ratios

PAM additions had the greatest effect on soil $\delta^{13}C$ and $\delta^{15}N$ values where background organic C content was lowest. For C, the highly negative $\delta^{13}C$ values (mean −29.3‰) of the added PAM caused a noticeable effect on low-C soils, but this effect was less obvious in the high-C soils. The high statistical correlations of $\delta^{13}C$ values with PAM enrichment in the Xeric Haplocalcid soil, but not the Typic Torripsamment, Typic Kandiudult, or Xeric Argialboll soils, may be related to the amount and form of organic C in these soils. The growth and addition of C and N via roots of C_3 pasture grasses can decrease the $\delta^{13}C$ and $\delta^{15}N$ in former C_4 cropland soils (Glaser, 2005; Rhoads et al., 2000; Jastrow et al., 1996). Fresh organic matter addition to agricultural, pasture and forest soils has been reported to lower soil $\delta^{13}C$ and $\delta^{15}N$ values while decomposition increases these values (Osono et al., 2007; Schweizer et al.,

Table 6
Concentration of anionic polyacrylamide (PAM) in a Durinodic Xeric Haplocalcid soil in Kimberly, Idaho as measured by $\delta^{13}C$ values

PAM loading (kg ai PAM ha^{-1})			
1995–2000	0	2691[2]	5382[2]
2000	0 e	1317 c	4675 a
2006	0 e	691 d	1466 b

[1]In each column, and row values followed by the same letter are not significantly different as determined by the least square means test ($p < 0.05$, $n=9$).
[2]Amount of anionic polyacrylamide added to soil annually from 1995 to 2000. Polyacrylamide was not added to control treatments.
[3]Detection limit for PAM in soil using both $\delta^{13}C$ and $\delta^{15}N$ is 10 mg ai PAM kg^{-1} soil.

1999; Wedin et al., 1995). Plant growth, organic matter input and turnover rates of different soil C components gives soils different $\delta^{13}C$ and $\delta^{15}N$ values. Progressive $\delta^{13}C$ enrichment of organic matter that has been frequently observed could be related to a gradual shift in the relative contributions of plant components in the residual organic matter (Ehleringer et al., 2000). Polyacrylamide concentration was more easily quantified in the Xeric Haplocalcid soils as compared to the Typic Torripsamment, Typic Kandiudult or Xeric Argialboll soils. Higher C concentrations in the Typic Torripsamment, Typic Kandiudult or Xeric Argialboll soils resulting from C input to soils from winter cover crops in addition to summer crops as compared to only summer crops growing in the Xeric Haplocalcid soil may have masked the lighter $\delta^{13}C$ and $\delta^{15}N$ that comprise the polyacrylamide molecule. PAM concentrations in soils measured by $\delta^{13}C$, correlated well between the two laboratories, but PAM concentrations in soils measured $\delta^{15}N$ did not correlate may indicate that $\delta^{13}C$ may be the more useful isotope to quantify the chemical. Further studies using $\delta^{13}C$ and $\delta^{15}N$ are necessary to determine the best stable isotope to measure PAM concentration in a specific soil type.

Aliphatic soil organic compounds increase in $\delta^{15}N$ as degradation progresses and $\delta^{15}N$ may be a robust measure of organic matter decomposition in soil (Kramer et al., 2003; Osono et al., 2007; Schweizer et al., 1999). Despite evidence that $\delta^{15}N$ accumulates in organic compounds during microbial processing the mechanisms of N fractionation remain unclear (Adams and Grierson, 2001). The laboratory experiment relating PAM concentration in the four soils with $\delta^{13}C$ and $\delta^{15}N$ values were not incubated and therefore did not allow PAM degradation. In the field, we measured only the amount of PAM in soil after 6 and 12 yr. In order to quantify $\delta^{13}C$ and $\delta^{15}N$ fractionation it would have been necessary to distinguish the $\delta^{13}C$ and $\delta^{15}N$ in soil microorganisms or in soil CO_2. This was beyond the scope of the study.

Soils with higher C or N concentrations did not show significant relationships between PAM concentrations and $\delta^{13}C$ values in the Typic Torripsamment, Typic Kandiudult or the Xeric Argialboll soil. The Xeric Haplocalcid soil formed in a xeric environment; it is a basic soil with a high concentration of Ca^{+2}. A modest amount of Ca^{+2} in the water shrinks the electrical double layer surrounding soil particles and bridges the anionic surfaces of soil particles and PAM molecules, enabling flocculation (Orts et al., 2001; Wallace and Wallace, 1996). Calcium ions act as a bridge between anionic soil surfaces and the anionic PAM macromolecule. The double charge and small hydrated radius of Ca^{+2} favors flocculation. By contrast, Na ions have a large hydrated radius which impairs ion bridging, generally leading to dispersion rather than flocculation of solids. The higher concentration of base cations, particularly Ca^{+2}, may have increased bonding of anionic polyacrylamide to soil particles and promoted a more thorough mixing of polyacrylamide in the Xeric Haplocalcid soil compared to the Typic Torripsamment, Typic Kandiudult or Xeric Argialbolls soils.

Anionic polyacrylamide may affect microbial growth by binding microorganisms to soil particles or to one another, restricting their mobility and access to C and nutrients or reducing N-fixing bacteria via nitrogen additions. The anionic PAM used in this study is nontoxic to most soil organisms (Barvenik, 1994; Seybold, 1994; Bologna et al., 1999). Sojka et al. (2006) found that active microbial biomass in these plots was 27–48% greater in these same untreated controls than soil treated with 2691 or 5382 kg ai PAM ha^{-1}. In addition, whole soil fatty acid methyl ester (FAME) profiles showed no discernible change in the soil microbial community due to either of the PAM treatments.

4.2. Polyacrylamide degradation rates in the field

When anionic PAM degradation rates were based on $\delta^{13}C$, we found that PAM degraded at a rate of 9.8% yr^{-1}. We incorporated 897 kg PAM ai ha^{-1} into the soil in the spring every year for 3 yr to plots receiving 2691 kg ai PAM applied ha^{-1} from 1998–2000. We found that Superfloc 836A PAM degrades at 9.8% yr^{-1}. When we incorporated 897 kg PAM ha^{-1} into the soil in the spring every year for 6 yr to plots receiving 5382 kg ai PAM applied ha^{-1} from 1995–2000 we found that for the first 6 yr Superfloc 836A PAM degraded at approximately 4.5% yr^{-1}. After 12 yr, in 2006, we found 1466 kg ai PAM indicating that PAM degraded at approximately 9.8% yr^{-1}. The slow degradation rates of PAM at high application rates may be the result of PAM clumping and therefore not being readily accessible to soil microorganisms. The 5382 and 2691 kg ai PAM ha^{-1} application rate was unreasonably high since PAM is traditionally applied at 1–2 kg ai PAM ha^{-1} per irrigation or 10–12 kg ai PAM ha^{-1} yr^{-1} (Sojka et al., 2007; 1998a; 1998b; Lentz et al., 2000; 1998).

Sojka et al. (2006) found that active bacterial biomass in these plots was 20–30 % greater in the control treatment than in soil treated with 2691 or 5382 kg ai PAM ha^{-1}. Active fungal biomass in soils was 30–50% greater in the control treatment than soil treated with 2691 or 5382 kg ai PAM ha^{-1}. Active microbial biomass in soil was 27–48% greater in the untreated control than soil treated with 2691 or 5382 kg ai PAM ha^{-1}. Whole soil fatty acid profiles showed no discernible change in the soil microbial community due to either of the PAM treatments at any sampling time (Sojka et al., 2006). Since Superfloc 836A PAM was expected to degrade at approximately 10% yr^{-1} in soil PAM was degrading each year as we added an additional 897 kg ai PAM ha^{-1}. Therefore PAM degradation calculations are not as simple as dividing the amount of PAM remaining in the soil by the number of years since the addition of the total amount of PAM. Our data agrees with results of Azzam et al. (1983). Because we have not considered PAM-specific carbon isotope fractionation effects, our PAM degradation rates may be overestimated. However, since the $\delta^{13}C$ measures all C with bulk soil samples the specific $\delta^{13}C$ in the sample, some of which may not be in the form of PAM, our degradation rates are more likely low because the measurement would also include mineralized C reincorporated into soil microorganisms and soil organic matter. Entry et al. (1992) found that as para-hydroxybenzoic acid and catechol was degraded, 15–21% of the ^{14}C labeled carbon was incorporated into soil fungal tissue. Further testing using ^{14}C or ^{13}C labeled anionic polyacrylamide is necessary to confirm this discrepancy.

4.3. Conclusions

Natural abundance of ^{13}C ($\delta^{13}C$) may be a viable technique for estimating the PAM concentrations in some soils with low-C content, though its calibration and reliability are soil dependent. The technique provides an analytical tool that can be used to estimate PAM degradation under various soil management regimes. This capability should be helpful in providing data to address environmental concerns regarding PAM accumulation and degradation in soil.

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