A correlation of $\delta^{15}N$ isotope ratios in wetland plants with NO$_3$-N in groundwater that receives nitrogen loading from fertilizer at a golf course in northern lower Michigan

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See separate discussion sections for Shannon and Liza

ABSTRACT

The correlation between nitrogen in groundwater and nitrogen in vegetation in a wetland receiving groundwater inputs was analyzed by measuring the stable isotope $\delta^{15}N$ in vegetation and the NO$_3$-N in groundwater on a golf course in northern lower Michigan. Through fertilization of a golf course green, nitrogen became enriched in the groundwater of an adjacent wetland. A grid with 52 data points was established on the wetland and groundwater and vegetative samples were collected from various points within the grid. Levels of NO$_3$-N in groundwater and $\delta^{15}N$ in shallow and deep-rooted plants were measured and compared to determine the correlation between groundwater nitrogen and the nitrogen that is taken up in plants. The groundwater nitrogen plume and enriched vs. unenriched plants were mapped in the wetland. $\delta^{15}N$ values in shallow-rooted plants varied widely and did not show a relationship between enriched $\delta^{15}N$ values in the plants and elevated NO$_3$-N in groundwater. Enriched $\delta^{15}N$ values in deep-rooted plants corresponded with elevated NO$_3$-N in groundwater, indicating that they take up nitrogen from groundwater. With further research it may be possible to use elevated levels of $\delta^{15}N$ in deep-rooted plants as an aid in mapping groundwater nitrogen plumes.

INTRODUCTION

Participation in golf has risen dramatically in North America in recent years, which has led to the construction of many golf courses in northern Michigan (National Golf Foundation, 2000). Golf course construction changes the hydrology and topography
of an area, which are important factors governing the quantity and chemistry of runoff to streams, lakes and groundwater (Winter and Dillon, 2005). These alterations are potentially detrimental to aquatic systems considering that turf grass establishment and maintenance require regular applications of fertilizers and pesticides (Shuman, 2002; Winter and Dillon, 2005). Applying fertilizers can result in the eutrophication of nearby water bodies, especially ones within or near a golf course’s boundaries (Kunimatsu et al., 1999). In general, pollutants from golf courses raise the nitrate levels of nearby streams through surface runoff and groundwater contamination (Mallin, 2000). The chemical impacts of golf course construction and operation are not well understood despite the promotion of “environmentally friendly” golf course design for the protection of water quality (Lewis et al., 2002). Research on the fate of fertilizer nutrients and the plumes they generate from turf grass runoff has been somewhat limited (Petrovic, 1990).

Though nitrogen can be tested in groundwater, the process can be time consuming and costly for golf courses. In order for a golf course to monitor the effects of fertilizer on the environment, easier and less costly methods are favored. One potential way of tracing nitrogen pathways is by using data from isotope ratios. $^{15}$N can be tested in vegetation. It is much easier and less expensive to collect plant tissue (leaves) than it is to collect groundwater samples. $\delta^{15}$N values can contribute to source-sink (tracer) information (Peterson and Fry, 1987). In other words, stable isotope ratios like $\delta^{15}$N can tell us about the source of nitrogen enrichment. It can tell us if nitrogen enrichment is coming from fertilizer applications or from some other source by comparing the isotopic ratios in the fertilizer to the isotopic ratios in plants. If the isotope ratios are similar, then there is a correlation between fertilizer and nitrogen enrichment.
A direct correlation between NO$_3$-N in groundwater and $\delta^{15}$N in plants is not known at this time. The purpose of this study is to propose a method of using plant tissue as a proxy to define a nitrogen plume in groundwater. We examine three questions. First, is there a nitrogen plume in the groundwater and if so, what is the extent of the plume? Second, is there a correlation between nitrogen enrichment in groundwater and enriched $\delta^{15}$N in plant tissue? Third, is fertilizer the source of nitrogen enrichment in the plants?

Two types of plant tissue (leaf tissue from deep-rooted and shallow-rooted plants) were tested for $\delta^{15}$N. If there was a correlation between nitrogen enrichment in groundwater and in plant tissue, we expected to find higher levels of $\delta^{15}$N in deep-rooted plants that correlate with high NO$_3$-N in groundwater because it is more likely that their roots will tap into groundwater and take up nutrients. We also expected to see low levels of $\delta^{15}$N values in the majority of shallow rooted plants because it is less likely that their roots will tap into groundwater and take up nutrients. Lastly, if the source of nitrogen is indeed from the fertilizer, we expected to see similar isotopic ratios of nitrogen between plant tissue and organic fertilizer. Organic fertilizer is more likely to have enriched $\delta^{15}$N values because it is made from animal by-products. Animals are in a higher trophic level and thus have magnified $\delta^{15}$N (Peterson and Fry, 1987). The nitrogen in inorganic fertilizers is in the form of urea, and is derived from nitrogen in air. Isotopic ratios for nitrogen in air are at or near zero, and are the standard by which all other ratios are compared.
METHODS

Study Site

Black Lake Golf Course (BLGC) is in Onaway, Michigan (Map 1). It is an 18-hole course that was designed to operate within the existing framework of the surrounding environment (Black Lake Golf Club, 2007). To maintain its initial goal of promoting an environmentally friendly golf course (though not required to by law), BLGC has been monitoring the impacts that fertilization on the golf course has had on the local environment. Their intent is to avoid adding any additional nutrients to the ponds and streams that flow through the course.

With the help of Michael Grant, a chemist from the University of Michigan Biological Station, BLGC started monitoring their groundwater in 1998 and several wells were established on the course (Map 2). Since 1998, Mr. Grant has closely monitored these wells, once in the spring and once in the fall of each year. Over the years, an elevation in NO$_3$-N, from $<0.5$ppm in 1998 to $>4.0$ppm in 2007, was observed in a monitoring well (MW-6) located in a wetland that receives drainage from the eighth green (Map 2 and Figure 1). However, the monitoring well MW-4 across the pond adjacent to a fairway showed no change in levels of nitrate (Map 2).

The eighth green overlooks a patch of wetland that drains into an adjacent pond (Maps 2 and 3). Underneath the surface of the green are three drainage tiles (with spaces in between them) that sit atop a thick layer of sand. This enables the green to be watered regularly and drain efficiently enough to keep the green dry. The drainage tiles drain into the wetland at three different points (Map 4). A stream flows through the pond and is adjacent to a small portion of the wetland (Map 2 and 3). There is a slope from the top of
the green, through the wetland, down toward the open water of the pond (Map 3). According to Mike Grant, surface water upstream and downstream of the wetland and pond have been tested for NO$_3$-N for several years and do not show signs of nitrogen input from the drains.

The wetland vegetation is characterized by *Typha* *spp.*, *Scirpus* *sp.*, grasses, rushes, *Alnus rugosa*, and ferns. There is a large *Typha* *spp.* stand within sites 32, 33, 34, 38, 39, and 40 (Map 4).

Nutrients from the fertilizers that are added to golf course greens can seep in between the drainage tiles and drain from the tiles out the drains into the groundwater table and flow into open water bodies. BLGC regularly applies four fertilizers: (1) Nature Safe 8-3-5 Stress Guard fertilizer, an inorganic solid fertilizer with 8% N content; (2) Lebanon Pro 25-3-10 fertilizer, an inorganic solid fertilizer with 25% N content; (3) Progressive Turf: Turf Foundation 10-3-5 fertilizer, an organic liquid fertilizer with 10% N content; (4) Progressive Turf: Greater Green 5-0-7 fertilizer, an organic liquid fertilizer with 5% N content. Fertilizers are regularly applied to all greens between April and November. 10-3-5 and 5-0-7 are applied bi-weekly while 8-3-5 is applied bi-monthly. 25-3-10 is applied twice a summer, in June and August.

Because nutrient loading from fertilizers contributes to eutrophication, it would be ideal to keep the fertilizer contained within the space that it is applied (Dodds, 2002). Once the elevated nitrogen was discovered and the threat of it leaving the wetland and flowing downstream was realized, the golf course wanted to investigate the situation further. They were interested in learning more about how much nitrogen input was going into the wetland, and if there were any patterns or plumes of nitrogen extending out from
the drains. Mr. Grant was also interested in mapping the plume, but collecting groundwater samples and having them analyzed can be time consuming and costly for a small golf course. He was interested in finding an easier way for golf courses to monitor nitrogen enrichment from fertilizers.

**Field methods**

A grid (56.1m deep x 36.0m wide) was established in an irregular-triangle shaped wetland that lay between the eighth hole of the BLGC golf course and a pond that received input from a stream (Map 2 and 3). The grid consisted of 7 rows and 7 columns with 52 data collection points arranged in quadrats. The quadrats in the first three rows were 8m wide and 4m deep. The quadrats in the last four rows were 8m wide and 5m deep. Using a combination of traditional methods (graph paper), GPS, and GIS, a map of the wetland was constructed (Map 4).

Groundwater was collected from each data point with a KV Associates Shallow Well Point Sampler. Two types of vegetation - one deeply rooted and one shallowly rooted plant - were collected from within a 1m radius of each data point (Map 4). Typical selections for deep rooted plants were *Typha spp.*, raspberry and fern. Typical selections for shallow rooted plants were grasses and sedges. Samples of fertilizer used at the golf course were also collected.

**Laboratory Methods**

Groundwater samples were filtered with a 4.5 micron acid washed filter and run through a Bran Leubbe Autoanalyzer III to determine levels of NO₃⁻-N. Vegetation samples were dried in a 60°C oven and finely ground with a Black and Decker Smart Grind coffee grinder. Vegetation and fertilizer were introduced into a Costech Elemental
Combustion System and transferred to a Thermo Finnigan Delta Plus XP mass spectrophotometer. The mass spectrophotometer tested for ratios of $\delta^{15}$N/$\delta^{14}$N and the ratios were compared to a standard (nitrogen gas in the atmosphere). Instrumental error was ±0.2.

**Mapping**

A map of the site and grid was created with ArcGIS (Map 4). The groundwater nitrogen plume was mapped according to site by site NO$_3$-N concentrations in the groundwater (Map 5). For the groundwater plume, values above 400ppb (with a range up to 4,500ppb) were considered “High”, values between 50ppb and 400ppb were considered “Medium”, and values below 50ppb were considered “Low”. The $\delta^{15}$N values for both deep and shallow-rooted vegetative samples were mapped separately and categorized as enriched or unenriched (Map 5). For both maps, $\delta^{15}$N < 3 are considered “unenriched” and values $\delta^{15}$N >= 3 are considered “enriched”. The groundwater and vegetative maps were compared to see if there was a correlation between the groundwater plume and the enriched vegetative samples.

**RESULTS**

The groundwater nitrogen plume was concentrated around the center of the wetland, with a few high spots at the edges (Map 5). The direction of the plume had a northward trend, which follows the topography of the area and moves toward the pond. The enriched deep-rooted plant tissue corresponded somewhat with the groundwater plume (Map 5). The majority of enriched plants were around the center of the wetland, and there were enriched spots on the outskirts of the wetland that corresponded with the high spots on the outskirts of the groundwater plume. The enriched shallow-rooted
vegetation varied more widely. While most of it was concentrated around the center of the wetland and there was one high spot on the outskirts, the concentration of enriched samples is not as dense as it is among the deep-rooted plants.

$\delta^{15}\text{N}$ values for the inorganic fertilizers were -0.2 and -0.5 for fertilizers 23-5-10 and 10-3-5, respectively (Table 1). Those values are very close to the $\delta^{15}\text{N}$ values in air. $\delta^{15}\text{N}$ values for the organic fertilizers were 4.5 and 2.1 for fertilizers 8-3-5 and 5-0-7, respectively. The weighted averages for the amount of $^{15}\text{N}$ applied to the golf course in the last 3 years were 1.1 $\delta^{15}\text{N}/100\text{ft}^2$ in 2005, 1.3 $\delta^{15}\text{N}/100\text{ft}^2$ in 2006, and 2.0 $\delta^{15}\text{N}/100\text{ft}^2$ in 2007. Since the golf course has yet to complete their fertilizing for the year, the delta units for 2007 represent only about 2/3 of the total number of fertilizer applications that will happen by the end of 2007.

**DISCUSSION (by Shannon)**

The correlation between high $\delta^{15}\text{N}$ in deeply rooted plants and high groundwater NO$_3$ indicates that these plants were getting nitrogen from the groundwater. We can assume that the groundwater has become enriched from the fertilizer that is applied on the green because data recorded from MW-4 showed 0.001ppm nitrate in 2006, while MW-6 showed 2.78ppm. MW-4 is in the rough next to the fairway across from the pond (Map 2). The fairway and green are fertilized differently. The green is fertilized more frequently throughout the year than the fairway. More fertilizer on the green would put more nitrogen into the groundwater.

We can also infer that the source of nitrogen is the fertilizer by comparing the $\delta^{15}\text{N}$ values in fertilizer with $\delta^{15}\text{N}$ values in plants. If the values are similar, it suggests
that the fertilizer is the source of the nitrogen. However, our values were slightly higher than the values in the fertilizer. The $\delta^{15}$N values for inorganic fertilizers were -0.2 and -0.5 for fertilizers 23-5-10 and 10-3-5, respectively (Table 1). These values are very close to zero and indicate that this fertilizer derived its nitrogen from air. $\delta^{15}$N values for the organic fertilizers were 4.5 and 2.1 for fertilizers 8-3-5 and 5-0-7, respectively. These values are above zero and indicate that the nitrogen in them was derived from animal byproducts. According to Coplen et al (2002), synthetic fertilizers have a minimum $\delta^{15}$N value of -3 and a maximum of +6. Biological fertilizer has a min $\delta^{15}$N value of +3 and a max value of +15. Our organic fertilizers were 4.5 $\delta^{15}$N and 2.1 $\delta^{15}$N. When we looked at the fertilizer ingredients we discovered that the $\delta^{15}$N were low because the nitrogen in the fertilizer was a mixture of organic and inorganic nitrogen. This explains why we found delta values lower than +15 in our vegetative samples.

We also think that denitrification is playing a role in nitrogen enrichment in the plants. The weighted averages of $\delta^{15}$N from fertilizer applied to the golf course over the last three years were 1.1/100ft$^2$, 1.3/100ft$^2$, and 2.0/100ft$^2$ for 2005, 2006, and 2007 respectively (Table 1). If fertilizer was the only source of $\delta^{15}$N, we would expect the plants and fertilizer to show similar $\delta^{15}$N. However, some of the delta values for the plants were as high as 9.6. This indicates that other factors come into play that are causing nitrogen enrichment in the plants. Since the plants are in a wetland, and wetland soils tend to be anoxic, we assume that denitrification is playing a major role.

Denitrification increases $\delta^{15}$N by 5-10 delta units (Peterson and Fry, 1987). When we combine the weighted average of $\delta^{15}$N for the fertilizer in 2007 (2.0) with the range of delta values that can be added through denitrification, the plants should have $\delta^{15}$N values
that range from 7-12. Our enriched plants fell within that range, which suggests that the plant enrichment is caused by a combination of fertilization and denitrification.

It is likely that the variability in shallow rooted plants is due to the variation in the wetland system. Shallow rooted plants receive more nutrients from run off than from groundwater. The wetland has a variety of microhabitats that contain different vegetation. There are some areas with standing water which contain *Typha spp.*, some drier, grassier areas, and some higher spots with sandy hills dominated by *Alnus rugosa*. These differing microhabitats will receive run off differently. Furthermore, the fertilizer is not applied consistently, but rather in bursts. So the surface of the wetland is receiving run off that contains bursts of nutrients. This may account for the variability of $\delta^{15}$N in the shallow rooted plants.

Further study needs to be done at this time to make a direct correlation between high groundwater nitrate and high $\delta^{15}$N in plants. However, there is great potential for a technique such as this to become a proxy for monitoring nutrient distribution at golf courses.

**Areas for further study**

Additional experiments that would make this relationship more clear would be to compare vegetation at two sites that are different from the site in this study – one from a nearby site where there was no fertilizer being added but denitrification (another wetland not adjacent to a managed part of the course) was occurring and a site where no fertilizer or denitrification was occurring. The $\delta^{15}$N values in plants at these two types of sites could be compared to the $\delta^{15}$N values in the plants in this study to get a better picture of the effects of denitrification in this wetland.
A profile of ground water pressure and flow would also provide more information about the movement of water at this study site. Though we assume that groundwater is flowing toward the pond (due north), we don’t know for sure if it is going due north or if it changes directions. The direction of flow could change depending upon whether or not the water has seeped in between the drainage tiles and into groundwater, or if it goes into the tiles and out the drains. If it goes out the drain, the direction of flow might change depending on which drain the water comes out of. A groundwater pressure profile would provide adequate information on the direction of flow and could explain why there are high levels of groundwater nitrogen in separate locations.

$\delta^{15}\text{N}$ values from plants at all the sampling sites would provide a more accurate picture of what is going on at the site. More data could provide a stronger (or weaker) correlation between $\delta^{15}\text{N}$ in plants and NO$_3$-N in groundwater. There are plans to test the rest of the plant samples at a later time. When this is done we will have a more accurate picture of what is going on at the site.

Other methods of isotope ratios have been used to map nitrogen plumes that may be useful in this case. One study measured nitrate in groundwater by using four different isotopes ($^{11}\text{B}$, $^{15}\text{N}$, $^{86}\text{Sr}$, and $^{87}\text{Sr}$) to measure the effects of buffering nitrate contamination in the groundwater (Widory et al., 2004). They used three other isotopes in addition to $\delta^{15}\text{N}$ because $\delta^{15}\text{N}$ tends to become elevated during denitrification (Widory et al., 2004). Denitrification makes it difficult to get an accurate value for $\delta^{15}\text{N}$ enrichment in fertilizer alone. $\delta^{15}\text{N}$ reflects both the source and the fate (i.e. denitrification) of NO$_3$–N in groundwater (Widory et al., 2004). Boron isotopes are not affected by denitrification, and thus will indicate the solute sources (Widory et al., 2004). But Boron
isotopes can also fractionate, so strontium is also used because it will not fractionate through any natural process (Widory et al., 2004).

Another study by Mankin (2000) simulated the effects of golf course development and management on ecosystems. They took fish habitats, native prairie grass, and avian habitats into consideration in their model. With this model, they found that, even with good management practices, annual nitrogen yield increased by 148%. With poor management practices annual nitrogen yield increased by 380%. These results suggest that other parameters can reflect what is occurring in a habitat besides ground water NO$_3$-N and vegetative sample $\delta^{15}$N readings.

In order to create a more accurate proxy for measuring nitrogen enrichment by using plant tissue vs. groundwater, it may be beneficial to incorporate several methods into the study to reduce the margin of error.

**DISCUSSION (Liza Wallis)**

$\delta^{15}$N values in enriched deep-rooted plants appear to be correlated with elevated NO$_3$—N concentrations in the groundwater of the wetland adjacent to the green. The enriched deep-rooted vegetation can be explained by anthropogenic factors, namely fertilizer applications of the adjacent green, and natural factors, largely denitrification when anoxic conditions are present.

Anthropogenic enrichment is apparent when $\delta^{15}$N values are greater than or equal to 3 (Brush and Elliott, 2006). When we mapped the level of $\delta^{15}$N enrichment in the deep-rooted vegetation, their values ranged from -4.5 to 9.6, indicating a source of enrichment in some areas of the wetland. The fertilizer was the likely source of anthropogenic
enrichment because the green drains directly into the wetland. BLGC uses a mixture of organic and inorganic fertilizers. The $\delta^{15}N$ values of their organic fertilizers are 2.1 and 4.5 (Table 1). These values are lower than the $\delta^{15}N$ values in biological fertilizers, which range from 3 to 15 (Coplen et al., 2002). The $\delta^{15}N$ values of their inorganic fertilizers are -0.2 and -0.5, which are lower than the $\delta^{15}N$ values of other synthetic fertilizers, which range from -3 to +6 (Table 1; Coplen et al., 2002). If the fertilizers were the only source of nitrogen, we would expect the range of $\delta^{15}N$ values in the vegetation to closely mirror the $\delta^{15}N$ values in the organic fertilizer. The weighted average of nitrogen from fertilizer ranged from 1.1 in 2005 to 2.0 in 2007, below the range of $\delta^{15}N$ values in the vegetation. Our results indicate that the fertilizer is a mixture of organic and inorganic fertilizer and that there is a source of nitrogen enrichment in addition to anthropogenic sources in the deep-rooted plants that we sampled.

The likely source of additional enrichment is the nitrogen produced by denitrification. When denitrification occurs, $\delta^{15}N$ values in plants range from 5 to 10 (Peterson and Fry, 1987). The range of $\delta^{15}N$ values in the deep-rooted vegetation that we sampled overlaps with the range of denitrification values, which suggests that denitrification is enriching some deep-rooted plants in the wetland. Denitrification can only occur in anoxic conditions where standing water is present for an extended period of time. Conditions favorable to denitrification occur in the wetland that we sampled for at least part of the growing season. The topography of the area is such that the wetland has areas of low elevation where water collects and creates waterlogged soils. Some of the deep-rooted plants that we sampled were in these low-lying areas where denitrification could have taken place. Our results indicate that we do not know the extent to which fertilization is
enriching the $\delta^{15}$N values in the vegetation, as these results appear to be partially the result of denitrification; therefore, we would not suggest using deep-rooted plants from the root depth at which we sampled to indicate elevated nitrogen levels in groundwater from fertilizer.

$\delta^{15}$N values in shallow-rooted plants do not show a strong correlation with elevated NO3—N concentrations in the groundwater, suggesting that the physical factors acting on shallow-rooted plants differ from those acting on deep-rooted plants. Shallow-rooted plants are affected greatly by surface runoff, which fluctuates depending on precipitation and topography of the immediately surrounding area. If shallow-rooted plants were only affected by surface runoff, we would expect to see enriched shallow-rooted plants aggregated at the green’s edge. There is enriched shallow-rooted vegetation extending throughout the wetland, however, indicating that surface runoff cannot be the only explanatory variable. Denitrification could be contributing nitrogen as well. Shallow-rooted plants would be affected by denitrification in those areas where standing water could be present. As the wetland approaches the pond, the elevation drops, and the surface and the water table have the greatest opportunity to interact. Those shallow-rooted plants that are closest to the pond and at the lowest elevation are most likely to exhibit nitrogen enrichment largely from denitrification (Map 4). This trend does appear to be evident (Map 5). There is enriched shallow-rooted vegetation in the areas of cattail stands where standing water was present and in those areas sampled near the pond’s edge. Our results indicate that the nitrogen sources acting on shallow-rooted plants are highly variable and more closely mirror the NO3—N concentrations of the surface water
rather than the groundwater. Shallow-rooted plants should therefore not be used to indicate levels of nitrogen enrichment in the groundwater.

**Areas for Additional Research**

Our sampling methods could be improved to obtain clearer and potentially more significant results. If we had used vegetation with roots that tapped into the groundwater for our deep-rooted samples, we could have more accurately known the nitrogen enrichment represented in vegetation by groundwater. The deep-rooted samples that we used indicated that there may be a correlation between nitrogen enrichment in deep-rooted plants and elevated NO$_3$—N groundwater concentrations, but they did not establish a significant relationship.

It would have been helpful to be able to test the groundwater samples for $\delta^{15}$N values rather than NO$_3$—N concentration. This could have allowed us to establish a firmer relationship between enriched vegetation and enriched groundwater.

Our selection of $\delta^{15}$N as the isotope that we used to measure enrichment was likely compromised due to denitrification. Other studies have successfully used isotopes such as Boron ($^{11}$B) and Strontium ($^{86}$Sr and $^{87}$Sr) in addition to $^{15}$N to map nitrogen plumes (Widory et al., 2004). These isotopes were chosen to account for the potential enrichment from denitrification. Boron isotopes serve as a good indicator of the enrichment source because they are not affected by denitrification. Boron isotopes can fractionate, however, and so Strontium was also used because it does not (Widory et al., 2004). This technique would be helpful in generating a more accurate map of the nitrogen plume from fertilizer enrichment.
ACKNOWLEDGEMENTS

We would like to thank; Mike Grant from the University of Michigan Biological Station for giving us the opportunity to be involved in this project, for giving us past monitoring well data, maps and blue prints, for analyzing our samples, and for his exhaustive help and expertise in the area of δ^{15}N; Bob Vande Koppel from the University of Michigan Biological Station for developing our maps and for his incredible patience; Doug Kendziorski for letting us work on the golf course and for providing us with golf carts and equipment; Chris Vogel for letting us use his Black and Decker Smart Grind coffee grinder that was custom fashioned for grinding leaves; Matt Pierle for his guidance, for his blood sweat and tears in the field, and for his help in the editing process; Eric Coleman for his help in the field and for grinding vegetation; Bob Pillsbury for connecting us to this project and for helping us with editing and statistics; and Summer Shandy and Miller Chill for helping us chill out.

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Map 5 There is some overlap between the plume of elevated nitrogen in groundwater and in the enriched N15 in deep rooted plants. Delta 15N values in shallow rooted plants are more variable.