CHAPTER 6. ISOTOPIC COMPOSITION OF NITRATE AS A DETERMINATION OF SOURCES OF POLLUTION

6.1. INTRODUCTION

A range of isotopic methods (using radioactive and stable isotopes) have been used to investigate groundwater processes and to determine the sources and pathways for groundwater contamination. Methods using stable isotopes of nitrogen have been applied in a variety of Australian and international groundwater studies. Isotopic methods have involved either application of tracer isotopes or studying natural variations in isotopic compositions. A variety of comprehensive texts outline applications of environmental isotopic methods in groundwater studies (Clark and Fritz 1997, Kendall and McDonnell 1998, Cook and Herczeg 2000, Hoefs 2004, Mook 2004).

While some isotopic methods are applied to directly investigate hydrological processes (such as recharge as reported in Chapter 4), other methods are particularly suited to understanding the origin and transport of contaminants. A number of studies have identified the isotopic fractionation of nitrogen through reaction and phase changes (such as evaporation) and this can allow the source identification of nitrate (Clark and Fritz 1997).

Application of nitrogen isotopic characteristics for determination of nitrate sources offers a number of advantages over other methods. Primarily, the advantages are that the method does not rely on identifying the source of the carrying water, but rather the nitrogen itself. While investigations into landuse change, recharge pathways and hydrochemistry can provide indications of potential sources of elevated nitrate concentrations in groundwater, the use of isotopic methods allows direct determination of the origin of individual nitrate ions (regardless of pathway or time).

Since the pioneering work of Amberger and Schmidt (1987), further work has demonstrated that the combined use of the oxygen and nitrogen isotopes of

nitrate may provide an improved ability to identify the source of nitrate contamination to groundwater.

While there have been a small number of studies in Australia using natural nitrogen isotopic composition for the identification of nitrate contamination in groundwater (Black and Waring 1977, Bolger and Stevens 1999, Changkakoti and Lawrence 1999, Harrington 1999), the use of the combined nitrogen and oxygen method does not appear to have been widely applied in Australia (no published studies were found). This chapter reports on an assessment of whether the origin of nitrate to groundwater could be determined through identification of the nitrogen and oxygen isotopic characteristics of nitrate, and whether this method identified different sources within the study area.

6.1.1 Nitrogen isotopes and environmental controls

Although there are a variety of radioisotopes of nitrogen (^{12}N, ^{13}N, ^{16}N, ^{17}N, ^{18}N and ^{19}N) these all have very short half-lives, ranging from 11 milliseconds to 9.97 minutes (Clark and Fritz 1997). The two stable isotopes of nitrogen are ^{14}N and ^{15}N, with a relative abundance in the atmosphere of 272 ($\pm$0.3):1 (Junk and Svec 1958), i.e. 99.63% of the nitrogen in air is ^{14}N.

The use of the nitrogen isotope in nitrate source identification is possible because isotopic fractionation occurs as a result of the processing of nitrogen within the nitrogen cycle; resulting in different nitrogen pools having slightly different isotopic ratios (Amberger and Schmidt 1987).

Given that the variation in $^{15}N/^{14}N$ ratios can be small, a standard approach is used for the comparison of the variations. This approach reports the difference (delta or δ) between the ratio of interest (such as for a water sample) and a benchmarked ratio. In the case of nitrogen ratios, the benchmark ratio is for N_2 in atmospheric air. The use of a standard benchmark ratio allows comparison between studies (and geographic areas).

Because variations in ratios may be small, the differences in ratios are reported in parts per thousand (permil or ‰) compared to the benchmark (equation 6.1; Clark and Fritz 1997).

$$\delta^{15}N_{sample} = \left(\frac{\left({}^{15}N/{}^{14}N\right)_{sample}}{\left({}^{15}N/{}^{14}N\right)_{reference}} - 1 \right) \bullet 1000 \text{ ‰ AIR} \qquad \text{Equation 6.1}$$

The equation includes the clarification that the reference abundance used is atmospheric N_2 (although the N_2 reference is not generally included).

The isotopic fractionation of nitrogen is a result of the 'isotopic effect' that occurs with different isotopes of the same element. In simple terms, lighter isotopes will form slightly weaker molecular bonds than heavier isotopes of the same element, due to the slight differences in mass and resulting vibration frequency (Hoefs 2004). Therefore in any chemical reaction, the lighter isotopes will tend to react more quickly (Clark and Fritz 1997).

Where the reaction does not result in a complete conversion of the reactant (source) material, the isotopic signature of the reactant will be different to the isotopic signature of the product. This resulting difference in isotope ratios is called 'isotope fractionation'.

6.1.2 The nitrogen cycle and isotopic influences

Given that the application of nitrogen isotopes to groundwater studies is due to the isotopic fractionation that occurs through the nitrogen cycle, it is important to describe the nitrogen cycle and isotopic influences within the cycle.

There is not one standard nitrogen cycle and its depiction is usually determined by the purpose of the discussion and the nature of the environmental system (e.g. closed/open, aquatic, terrestrial, drought-affected, agricultural; Sprent 1987). For the purposes of this study, a simplified cycle applicable to the land systems and hydrogeology of the study area was adopted (Figure 6.1).

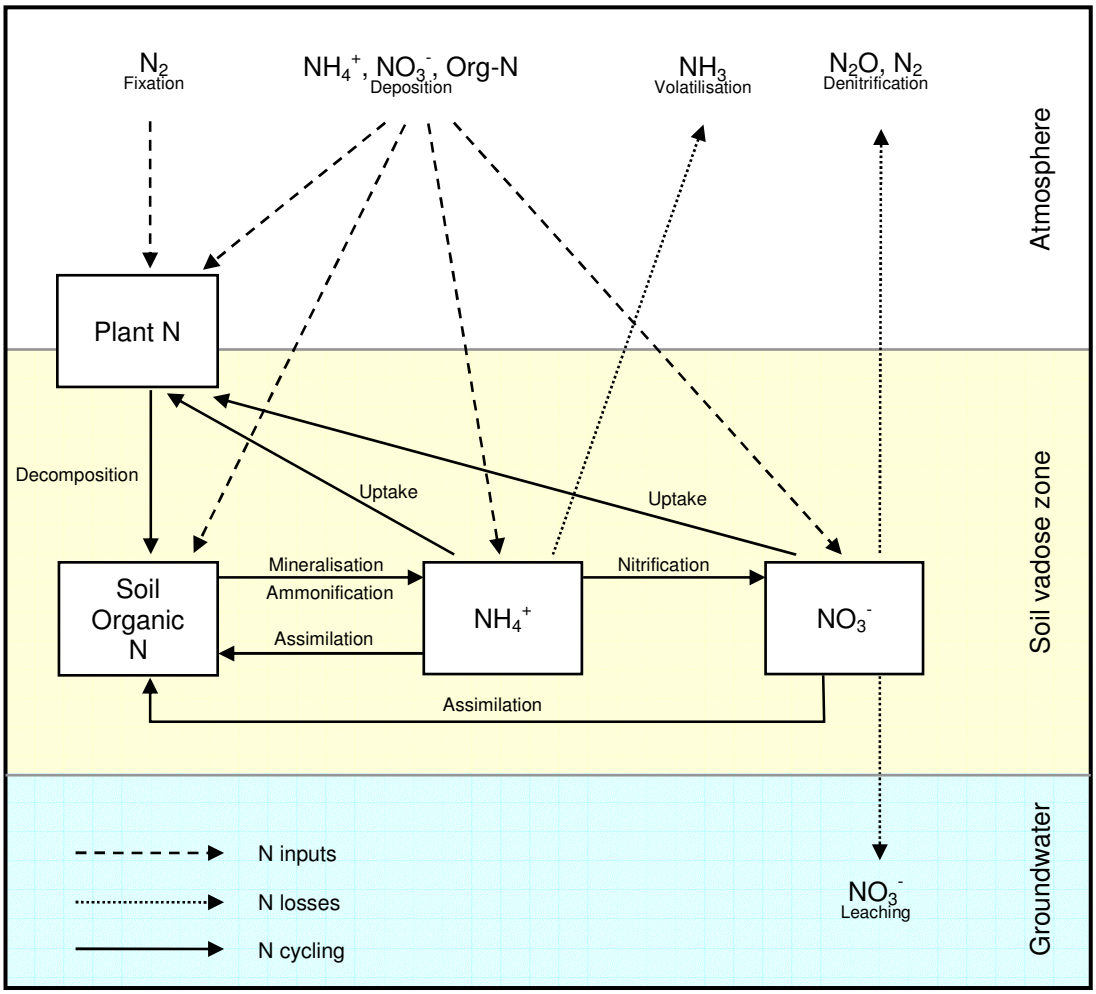

Figure 6.1: A simplified nitrogen cycle for the study area, adapted from Payne (1981), Sprent (1987) and Kendall (1998)

Each stage of the nitrogen cycle and its isotopic fractionation is described below. Further details are provided in Hübner (1986), Shearer and Kohl (1986), Högberg (1997) and Robinson (2001).

Nitrogen fixation ($N_2 \rightarrow NH_3$)

Nitrogen fixation is the conversion of atmospheric dinitrogen into ammonia. This process can occur biologically, through industrial processes (such as the production of fertiliser) or as a result of thunderstorms (Sprent 1987). Biological nitrogen fixation is the focus of this discussion.

Biological nitrogen fixation requires an initial energy input to dissociate the strong N≡N molecular bond, and this is only able to be undertaken by a

relatively select number of micro-organisms possessing the *nitrogenase* enzyme. These include certain cyanobacteria (aquatic, marine and terrestrial) and symbiotic bacteria associated with leguminous pasture plants (e.g. *Bradyrhizobium* or *Rhizobium*; Sprent 1987).

A variety of studies have identified that the biological fixation of N_2 slightly preferences the lighter nitrogen isotopes, resulting in a reported range of δ of -5 to 2‰ for fixed nitrogen (Delwiche and Steyn 1970, Létolle 1980, Högberg 1997, Kendall 1998). In practice, the fixation of N_2 is reported as resulting in no discernable fractionation (Shearer and Kohl 1990, Clark and Fritz 1997, Aranibar et al. 2003).

Mineralisation/Ammonification (Organic N→NH_4^+)

The mineralisation process is the conversion of complex organic nitrogen to ammonium; it is also referred to as ammonification. This decay process is undertaken by a variety of micro-organisms that utilise the carbon within the organic source and subsequently release ammonium (Sprent 1987).

Mineralisation usually results in a negligible fractionation between the soil organic matter and the produced ammonium (Högberg 1997, Kendall 1998).

Nitrification (NH_4^+→NO_2^-→NO_3^-)

Nitrification is a multi-step oxidation process for the conversion of ammonium into nitrate (although it is usually described as two stages). The conversion of ammonium to nitrite and then to nitrate is controlled primarily by two metabolically independent groups of aerobic bacteria (Payne 1981).

The first stage of nitrification (oxidation of ammonium to nitrite; equation 6.2) is primarily driven by the *Nitrosomonas* bacteria and is usually the rate-determining step of nitrification (Kendall 1998).

$$NH_4^+ + \tfrac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad \text{Equation 6.2}$$

The second stage is the further oxidation of nitrite to nitrate (equation 6.3). This reaction is carried out by *Nitrobacter* and often occurs rapidly so that nitrite is not often detected in large concentrations in natural environments (although anaerobic or heavily contaminated sites may have high concentrations of nitrite).

$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^- \qquad \text{Equation 6.3}$$

The isotopic fractionation of nitrogen through this process will depend upon the available organic nitrogen and the environmental conditions of the soil. In a (relatively) undisturbed environment at equilibrium, any fractionation will be the result of the slower oxidation step of the *Nitrosomonas* (as the nitrite is subsequently entirely consumed there is no fractionation). However, the degree of fractionation may be impacted considerable, or vary over time, in systems receiving large depositions of nitrogen (such as ammonium fertiliser); (Högberg 1997, Kendall 1998). The fractionation factors (δ) for the nitrification process vary from 0‰ to -40‰ (Shearer and Kohl 1990, Kendall and Aravena 2000), with the narrower range of -12‰ to -29‰ being more recently accepted (Kendall 1998).

Assimilation and Uptake

Assimilation refers to the incorporation of nitrogen-based compounds (predominantly nitrate, nitrite and ammonium ions) into organisms (including plants). The assimilation of nitrogen-based compounds is a continuous recycling process of oxidation and reduction of nitrogen for the growth of these organisms (the mechanisms are too diverse to describe here).

Some high fractionation factors have been reported in studies investigating the assimilation of nitrogen compounds in aquatic environments (-27‰), however values of -2‰ to 1‰ are considered realistic for natural soil conditions (Shearer and Kohl 1986, 1990, Kendall and Caldwell 1998, Kendall and Aravena 2000).

Volatilisation

Volatilisation is the loss of ammonia gas, and may occur through the process of oxidation of some organic nitrogen sources (such as manures) and other ammonia based compounds (such as urea). Volatilisation of ammonia results in nitrogen loss from broad-acre application of urea as a fertiliser (and so discourages its use).

The volatilisation of ammonia can result in the $\delta^{15}N$ of the remaining manure piles being greater than 20‰ (Kendall 1998). A variety of measured fractionation factors are expected given the dependence of volatilisation on environmental conditions. A fractionation factor of -29‰ is reported by (Högberg 1997).

Denitrification

Denitrification is a multi-step process where nitrate is chemically or biologically reduced to dinitrogen (and nitrous oxide); in natural soil systems this N_2 (and N_2O) may be lost as gas from the system. The key driver for denitrification is that it takes place under anaerobic conditions (Sprent 1987), and although the soil profile may be aerobic, denitrification may occur in localised 'microsites' in soil or groundwater where anaerobic conditions exist (Koba et al. 1997).

The denitrification process has at least four identifiable steps, each controlled by a specific range of micro-organisms ($2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2$), each with the common goal of using the nitrogen as the terminal electron acceptor for anaerobic respiration (Payne 1981). The full process of denitrification is usually represented as the simplified equation 6.4.

$$4NO_3^- + 5C + 2H_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2 \qquad \text{Equation 6.4}$$

Denitrification is of particular interest in nitrogen isotopic studies as the process favours the lighter nitrogen isotopes, resulting in an exponential increase in the $\delta^{15}N$ of the remaining nitrate in the system as its concentration decreases. It is particularly relevant as once nitrate is formed

in the soil, denitrification and mixing are the dominant processes that will alter the isotopic composition of nitrate in groundwater.

The fractionation factor of denitrification ranges up to -40‰ (Shearer and Kohl 1990, Högberg 1997, Kendall 1998).

Deposition

The process of deposition has been shown generally in the simplified nitrogen cycle of Figure 6.1. In the study area, deposition is likely to be dominated by the field application of organic and inorganic sources of nitrogen (e.g. industrial wastewater, fertiliser, domestic wastewater), compared to other airborne nitrogen sources (e.g. off-site windborne, pollution generated, lightning generated). This assumption is supported by a previous local study. During 1974-1975, Blackburn and McLeod (1983) measured the mean concentration of nitrate in rainwater at Mount Gambier (which is subject to greater industrial air emissions of nitrogen than the study site). Their results were that the nitrate concentration was between 0.05 and 0.87 mg/L, with a predicted rate of deposition of 3.38 kg N/ha/yr. This is considerably less than deposition in more industrialised areas such as in the UK where between 10 and 20 kg N/ha/yr is reported (Wakida and Lerner 2005). For this reason it is not considered that atmospheric deposition will alter the isotopic characteristics of groundwater nitrate for the study area, even if it had a significantly different isotopic signature.

The isotopic characteristics of the main deposition sources are described in the later sections.

6.1.3 The Oxygen isotopes

As is the case with nitrogen, oxygen has a number of short-lived radioisotopes (^{12}O, ^{13}O, ^{14}O, ^{15}O, ^{19}O and ^{20}O); having half-lives of between 13.5 and 122 seconds (Clark and Fritz 1997). The three stable isotopes of oxygen, ^{16}O, ^{17}O and ^{18}O, have the proportional abundance of 0.97757, 0.00038 and 0.00205 respectively (Coplen et al. 2000), with the relative

isotopic ratio of $^{18}O/^{16}O$ being adopted widely for hydrological studies.

Application of the $^{18}O/^{16}O$ ratio in hydrological studies is possible due to the differential fractionation of oxygen isotopes throughout the hydrological cycle. This fractionation occurs initially through the preferential evaporation of the lighter water isotope from the world ocean (e.g. $^{1}H_2{}^{16}O$) due to it having a higher vapour pressure than the heavier isotope water molecules (e.g. $^{1}H_2{}^{18}O$ or even $^{2}H_2{}^{16}O$; Hoefs 2004). This fractionation occurs further during precipitation, where condensation (rain) tends to favour the heavier isotopes of water, thereby further depleting the ^{18}O of the remaining water vapour in the cloud system (Clark and Fritz 1997). The degree of depletion of ^{18}O in rainfall will depend on the geographic, climatic and meteorological conditions of the area.

Once rainfall reaches the land surface, it may infiltrate or be subject to further enrichment of ^{18}O as a result of evaporation from soil or from waterbodies. There does not appear to be any fractionation of oxygen during uptake by terrestrial plants, although transpiration within plants may result in enriched ^{18}O within the plant tissue (Dawson and Ehleringer 1998).

Based upon the early work of Craig (1961), the reference ratio for $^{18}O/^{16}O$ is a standard based upon ocean water and called the Vienna Standard Mean Ocean Water, or VSMOW. The calculation of the change in relative ratio is the same approach as has been described for $\delta^{15}N$ in equation 6.1, namely;

$$\delta^{18}O_{sample} = \left(\frac{\left(^{18}O/^{16}O\right)_{sample}}{\left(^{18}O/^{16}O\right)_{reference}} - 1 \right) \bullet 1000 \text{ ‰ VSMOW} \qquad \text{Equation 6.5}$$

While the $\delta^{18}O$ of the ocean is considered to be approximately 0‰ (VSMOW), the $\delta^{18}O$ of rainfall in the study area is expected to be between -3‰ and -5‰. This is based upon the mean $\delta^{18}O$ in rainfall of -3.8‰ for Adelaide (*n*=113, SD=2.4), and a mean $\delta^{18}O$ in rainfall for Melbourne of -4.6‰ (*n*=325, SD=2.0) between 1960 and 1998 (IAEA/WMO 2004).

In groundwater studies, the use of the $^{18}O/^{16}O$ ratio has increased functionality, as there are other processes that can lead to further changes of oxygen isotopes in water. These include;

1. recharge from surface waters that have been subject to further evaporation will have different $\delta^{18}O$ characteristics,
2. recharge that has occurred in the past (where different ambient temperatures resulted in different isotopic compositions of rainfall) can be differentiated from more recent recharge,
3. in-aquifer (or in-soil) isotopic fractionation can illustrate kinetic or exchange reactions within the geological formations (Hoefs 2004).

The $^{18}O/^{16}O$ ratio of groundwater has been used within the Gambier Embayment of the Otway Basin to investigate groundwater recharge and flow mechanisms (Turner 1979, Allison and Hughes 1983, Turner et al. 1983, Love, et al. 1994, Leaney, et al. 1995, Herczeg, et al. 1997).

Atmospheric oxygen (O_2) has a constant $\delta^{18}O$ of 23.5‰ (Dole et al. 1954, Hoefs 2004).

6.1.4 Application of oxygen isotopes (of nitrate)

Amberger and Schmidt (1987) were perhaps the first to recognise that consideration of the oxygen (as well as the nitrogen) isotope ratio of nitrate could provide additional information on the source of nitrate in groundwater. In the same way that nitrogen isotope ratios can discriminate between sources, the oxygen isotope ratios can differentiate sources that would otherwise have overlapping isotope signatures.

Relevance to nitrification

The nitrification process oxidises ammonium into nitrate, however the industrial production of nitrate (for fertiliser) is different from the microbial process governed by the *Nitrosomonas* and *Nitrobacter* bacteria. The industrial production of nitrate results in most of the oxygen atoms being

sourced from atmospheric O_2, and therefore the $\delta^{18}O$ of this nitrate approaches that of atmospheric O_2. Amberger and Schmidt (1987) postulated that one sixth of the oxygen atoms for fertiliser production were sourced from water, hence resulting in $\delta^{18}O$ of between 18.7‰ and 22.7‰.

By comparison, the microbial nitrification process sources two oxygen atoms from water, and one from atmospheric oxygen (Andersson and Hooper 1983, Kumar et al. 1983). For groundwaters that have not been subject to substantial evaporative or geological processes, the $\delta^{18}O$ of nitrate would be between -10 to 10‰ (Kendall and Caldwell 1998). Therefore, the use of $\delta^{18}O$ to differentiate these different sources may be particularly useful where the $\delta^{15}N$ of the nitrate cannot discriminate between sources.

Relevance to denitrification

As with isotopic fractionation with nitrogen, the denitrification process also favours the lighter oxygen isotopes (Kendall 1998). A few studies have been undertaken to determine the fractionation that occurs during denitrification, and these report a predictable ratio of $\delta^{15}N$ enrichment compared to $\delta^{18}O$ of between 1.3 and 2.1 (Böttcher et al. 1990, Aravena and Robertson 1998, Kendall 1998, Cey et al. 1999, Mengis et al. 1999, Devito et al. 2000, Chang et al. 2002, Fukada et al. 2003, Fukada et al. 2004). A review by Chen and MacQuarrie (2005) of many of these studies resulted in them proposing a theoretical fractionation ratio of 1.96 (i.e. $\delta^{15}N$: $\delta^{18}O$ of 1.96:1).

Therefore, while the $\delta^{15}N$ value of groundwater may provide an indication of denitrification, the incorporation of the $\delta^{18}O$ data can provide greater confidence where this is suggested.

6.1.5 $\delta^{15}N$ and $\delta^{18}O$ of nitrate sources and pools

Despite the range of fractionation processes that may affect the isotopic signatures of both the oxygen and nitrogen of nitrate (including their unavoidable recycling through the nitrogen cycle), it is relevant to provide a description of the expected isotopic signatures of those sources that are

likely to be relevant to the study area (high arctic snow, desert depositions, marine, munitions and geological sources are not included). The data is provided in Appendix 7, and is presented graphically in Figures 6.2 and 6.3.

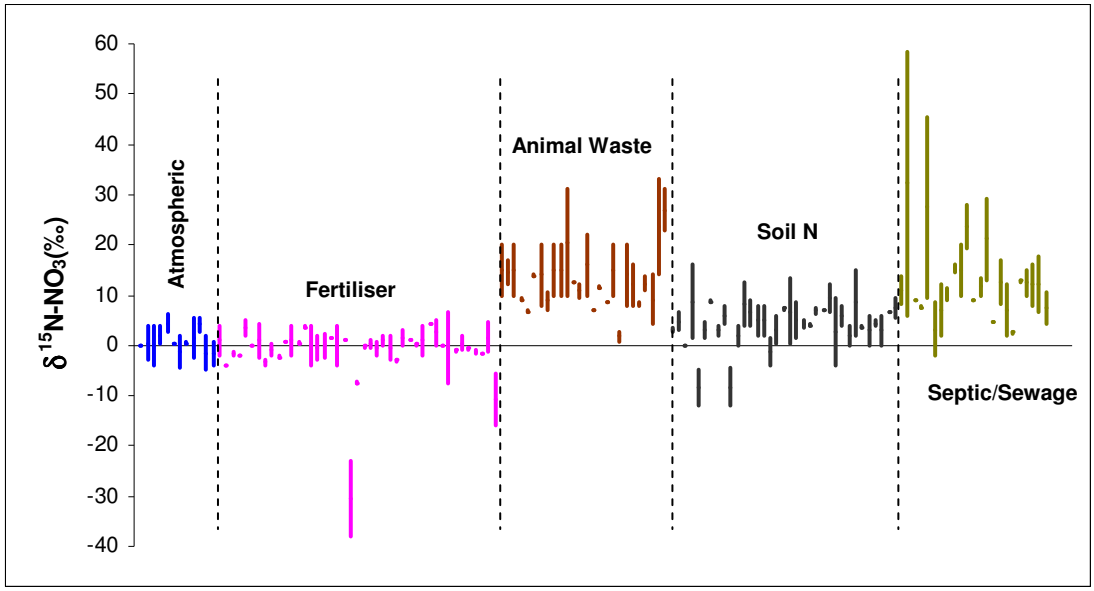

Figure 6.2: The $\delta^{15}N$ of nitrate from different sources from literature summarised in Appendix 7

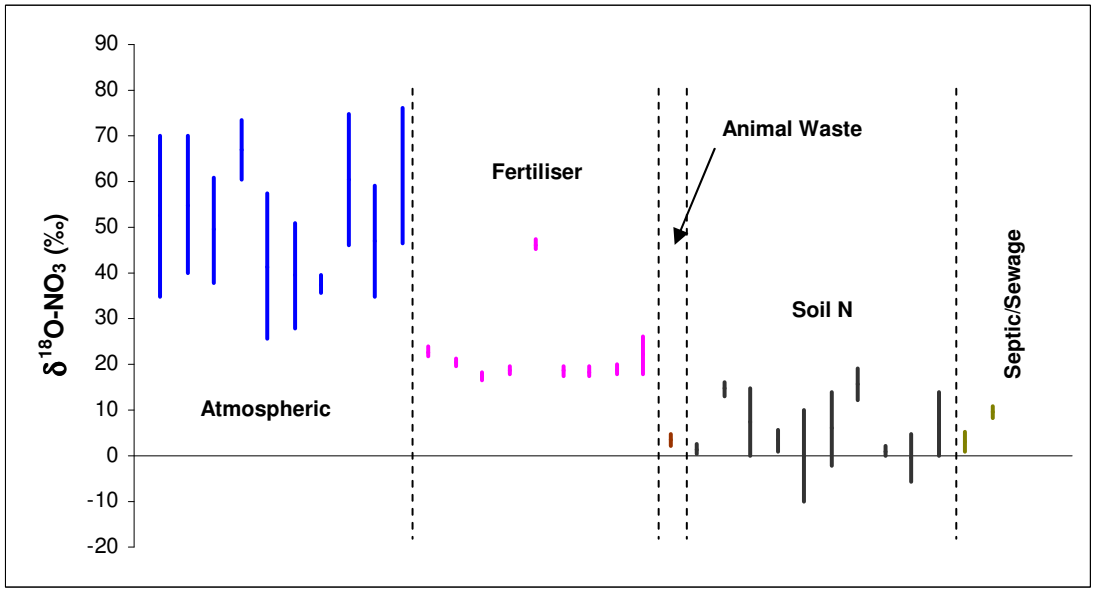

Figure 6.3: The $\delta^{18}O$ of nitrate from different sources from literature summarised in Appendix 7

Figures 6.2 and 6.3 illustrate that even allowing for considerable variation in measured isotope ratios of nitrate in the environment, there is distinct variation between the main nitrate sources to groundwater. Kendall (1998) recognised the distinction that could be achieved through this combined isotopic method, and Figure 6.4 is constructed based upon the methodology provided by her and using the updated literature data presented in Appendix 7.

While some studies in Appendix 7 have reported isotopic compositions outside the ranges shown in Figure 6.3, the combined use of the $\delta^{15}N$ and $\delta^{18}O$ of nitrate has the capacity to provide considerable guidance as to the source(s) of nitrate in groundwater systems.

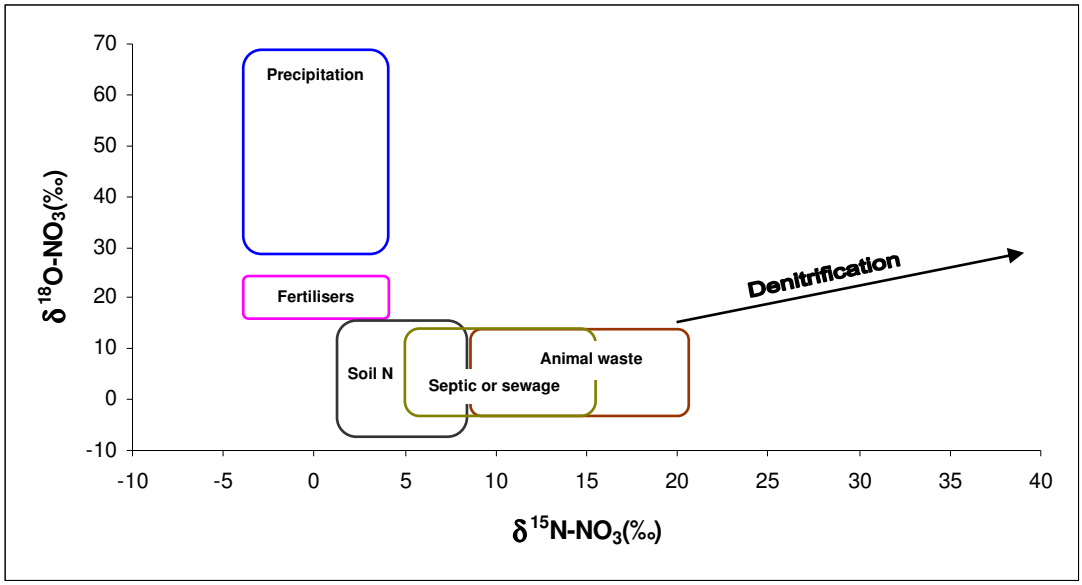

Figure 6.4: Schematic representation of nitrate sources based upon the $\delta^{15}N$ and $\delta^{18}O$ signatures

6.2. METHODOLOGY

Groundwater samples were collected from 33 bores within the study area for isotope analysis. These bores were specifically selected to sample the range of landuses and hydrogeological settings of the study area. Further, care was taken to ensure that those bores selected were suitably constructed, as

earlier work had identified that some bores allowed ingress of surface water. As the intention of the sampling program was to identify the source of nitrate in the groundwater, selection preference was towards those bores that were identified as demonstrating elevated nitrate concentrations.

Two nested wells were installed on the western portion of the study area (702306388, 702306389, 702306390 and 702306391) so that one well at each nest intersected the top three metres of the aquifer, and the other would intersect the top nine metres. Analytical results for these wells were included in source-determination analysis, but the sample size was insufficient to allow for assessment of vertical variability in nitrogen isotopes.

All samples were collected during October 2005 in accordance with the Murray-Darling Basin Groundwater Quality Sampling Guidelines (MDBC 2002). All bores were purged of at least three bore-volumes prior to samples being collected. The purging and pumping (for sample collection) of the wells was undertaken using either the pump installed at the well (for equipped wells) or using a low flow submersible electric pump or polyethylene bailer (for unequipped wells).

The physical and chemical water quality parameters (i.e. pH, temperature and electrical conductivity) were monitored during purging using a Model 611 Yeo-Kal water quality instrument (Yeo-Kal Electronics, Brookvale, NSW, Australia) until they were considered stable.

Samples from each well were collected in 600 mL PET plastic bottles, chilled, and forwarded to the Australian Water Quality Centre for analysis of TKN; (Method 4500-N-Org-D; APHA 1998), nitrate (Method 4500-NO_3-F; APHA 1998) and nitrite (Method 4500-NO_2-D; APHA 1998). This laboratory is NATA accredited for these analytical methods.

Samples for isotope analysis were filtered (0.45 µm filter) into 60 mL HDPE plastic bottles. Samples were preserved using 10M hydrochloric acid to lower pH to 2.5 to prevent biological transformation of nitrate during

transport. Samples were refrigerated and nitrogen and oxygen isotopes were analysed at the Woods Hole Oceanographic Institute (USA) using the microbial denitrifier method (Sigman et al. 1997, Casciotti et al. 2002).

Where samples were collected from unequipped bores, all sampling and pumping equipment was decontaminated with water which had a residual chlorine concentration above 0.2 mg/L to kill any potential iron bacteria and prevent cross-contamination of bores.

6.3. RESULTS

The results of the isotopic analysis of the sampled wells within the study area are presented in Table 6.1. Data is presented for ion concentrations and isotopic ratios (including standard deviations in brackets).

Due to the low nitrate concentrations in six wells, isotopic analysis was not reliable and these were not analysed further.

Figure 6.5 applies the methodology of Figure 6.4 to the isotopic composition of nitrate in groundwater in the study area.

The isotopic results indicate that the nitrate in groundwater is not directly derived from leaching of fertiliser or from precipitation. If either of these sources were the case, the $\delta^{18}O$ of nitrate would be expected to be considerably higher (above +18‰; see Figure 6.4). The highest $\delta^{18}O$ of nitrate recorded is +10.5‰, however this is associated with the highest $\delta^{15}N$ value of nitrate, which is well above the literature values for commercial fertilisers or precipitation sources.

6.3.1 Source Determination

Various papers highlight that while the nitrate isotopic signature of fertiliser is relatively predictable, those fertilisers containing ammonia will be the subject

Table 6.1: Isotopic analysis of wells within the study area

Bore Number	$NO_3 + NO_2$ (as N) (mg/L)	Total N (as N) (mg/L)	$\delta^{15}N\text{-}NO_3^-$ (‰)	$\delta^{18}O\text{-}NO_3^-$ (‰)
702300028	23.1	23.1	5.1 (0.13)	4 (0.07)
702300092	27.8	27.8	5.9 (0.09)	3.9 (0.22)
702300621	23.9	23.9	4.3 (0.04)	3.7 (0.06)
702300905	43.5	43.5	10.1 (0.17)	2.8 (0.09)
702300910	24.1	24.1	9.4 (0.1)	3 (0.23)
702300911	35.1	35.1	12.8 (0.11)	0.9 (0.24)
702301359	13.8	13.8	5.6 (0.15)	4.1 (0.07)
702301888	14.2	14.2	10.3 (0.02)	6 (0.21)
702302826	0.2	0.2	17.5 (0.11)	10.5 (0.13)
702302827	0.083	1.1	-	-
702302828	11.4	11.4	5.2 (0.07)	4.4 (0.04)
702302829	13.8	13.8	4.9 (0.03)	3.2 (0.18)
702302981	37.8	37.8	13 (0.12)	2.6 (0.24)
702302998	0.005	0.05	-	-
702303000	10.4	10.4	4.9 (0.01)	3.6 (0.11)
702303605	12.8	12.8	4.7 (0.17)	4.1 (0.08)
702303606	27.6	27.6	4 (0.16)	3.6 (0.18)
702303608	26.2	26.2	7.6 (0.13)	4.9 (0.19)
702303624	16.0	16.0	9.6 (0.12)	5.4 (0.14)
702303762	0.008	0.13	-	-
702303763	7.1	7.1	6.5 (0.04)	4.7 (0.06)
702303764	0.024	1.190	-	-
702303765	0.078	0.200	-	-
702303766	20.1	20.1	5.3 (0.09)	2.3 (0.03)
702303767	0.014	0.080	-	-
702304416	11.1	11.1	4.9 (0.03)	3.6 (0.27)
702305190	34.9	34.9	11.3 (0.04)	5.4 (0.03)
702305195	14.7	14.7	4.6 (0.02)	4.2 (0.12)
(Duplicate)			4.3 (0.1)	4 (0.36)
702305732	32.6	32.6	4.1 (0.06)	2.9 (0.18)
702306388	13.5	15.1	6 (0.18)	4.4 (0.03)
702306389	12.1	12.1	6.4 (0.2)	4.5 (0.15)
702306390	13.1	13.1	4.6 (0.15)	4 (0.11)
702306391	16.9	17.0	4.1 (0.17)	3.4 (0.05)

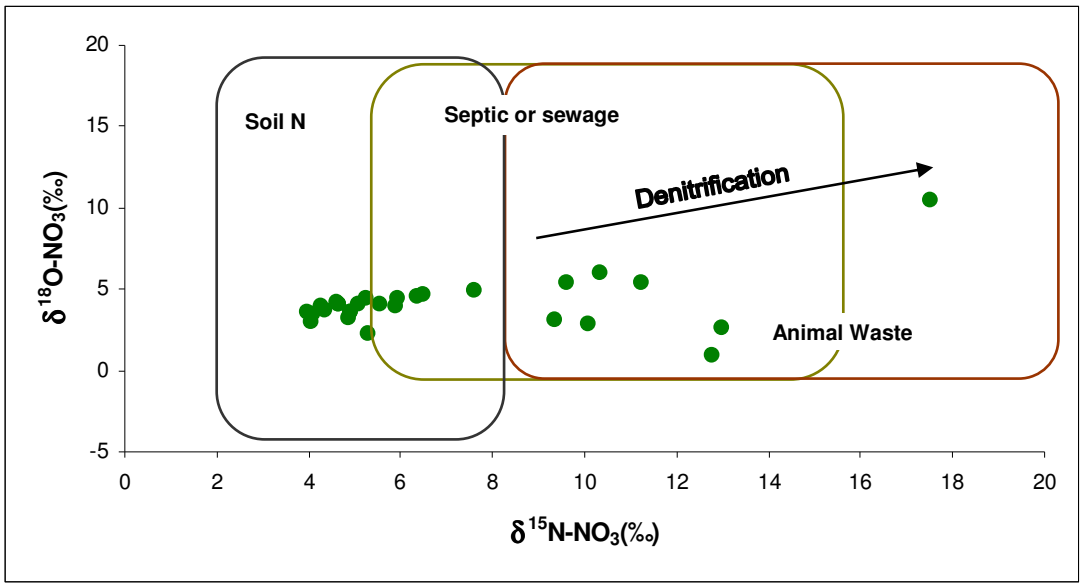

Figure 6.5: **The $\delta^{15}N$ and $\delta^{18}O$ data for groundwater samples from within the study area plotted against literature values and showing the denitrifying trend line**

of microbial nitrification within the soil profile (Black and Waring 1977, Amberger and Schmidt 1987, Wilson et al. 1994, Bálint et al. 2002, Bengtsson et al. 2003). As discussed above, the three oxygen atoms required for microbial nitrification of ammonia are sourced from water (two oxygen atoms) and from atmospheric diatomic oxygen (one oxygen atom); (Andersson and Hooper 1983, Kumar, et al. 1983). The $\delta^{15}N$ of nitrate resulting from the nitrification of ammonia is also reported to be less than for non-ammonia fertiliser due to the slightly lower average $\delta^{15}N$ of ammonia (in fertiliser), and the fractionation during microbial nitrification (Hübner 1986). As a result, nitrate from microbial nitrification of ammonia from fertiliser has isotopic ratios between approximately -5‰ and +4‰, and +4‰ to +11‰ (for $\delta^{15}N$ and $\delta^{18}O$ respectively; Clark and Fritz 1997). This 'source' of groundwater nitrate is not included on Figure 6.4 or 6.5 due primarily to the uncertainty associated with the ranges presented (the data ranges are presented in Appendix 7).

It is possible to calculate the theoretical $\delta^{18}O$ of nitrate resulting from microbial nitrification using the average $\delta^{18}O$ of groundwater and the

expected $\delta^{18}O$ of atmospheric air (Panno et al. 2001). In the study area, the $\delta^{18}O$ of groundwater is expected to be -4.5‰ (*n*=13);(Love, et al. 1992), and the atmospheric $\delta^{18}O$ is estimated to be +23.5 (Dole, et al. 1954, Hoefs 2004). While the theoretical value of the $\delta^{18}O$ of nitrate from microbial nitrification would therefore be +3.9‰, the actual (measured) $\delta^{18}O$ of nitrate is often higher by 5 to 10‰ for reasons that are not clear (Kendall 1998). In the case of the study area, the range of $\delta^{18}O$ of nitrate from microbial nitrification may therefore be slightly wider than found in literature (i.e. approximately +4‰ to +14‰).

As shown in Figure 6.5, all isotope results are within the literature range for soil generated nitrate or nitrate derived from septic or animal waste. The discrimination of these sources based upon nitrogen isotopes is difficult due to the overlapping values reported in literature (Figures 6.2 and 6.3). Landscape characterisation methods offer a means of determining potential nitrate source to groundwater, and this approach was applied.

In the study area, well locations were classified based upon the primary landuse in their immediate surroundings (Figure 6.6). The classification groups used were those reported in Appendix 7, based upon the information on surrounding landuses collected during field work. The isotopic composition of nitrate sampled from wells across the study area is illustrated in Figure 6.7.

The results indicate a spatial relationship for the oxygen and nitrogen isotope composition of the nitrate, and this is confirmed through a plot of the $\delta^{15}N$:$\delta^{18}O$ (Figure 6.8).

These figures (particularly Figure 6.8) demonstrate that nitrate isotope ratios correspond to those presented in literature when classified by potential local source. Further, the isotope ratios for nitrate from soil nitrification are significantly different from the nitrate isotopes predicted to be sourced from septic waste (mean $\delta^{15}N$=5.2, SE 0.2, $\delta^{18}O$=3.9, SE 1.4 (*n*=19) compared to mean $\delta^{15}N$=10.9, SE 0.6, $\delta^{18}O$=3.5, SE 0.7 (*n*=6)).

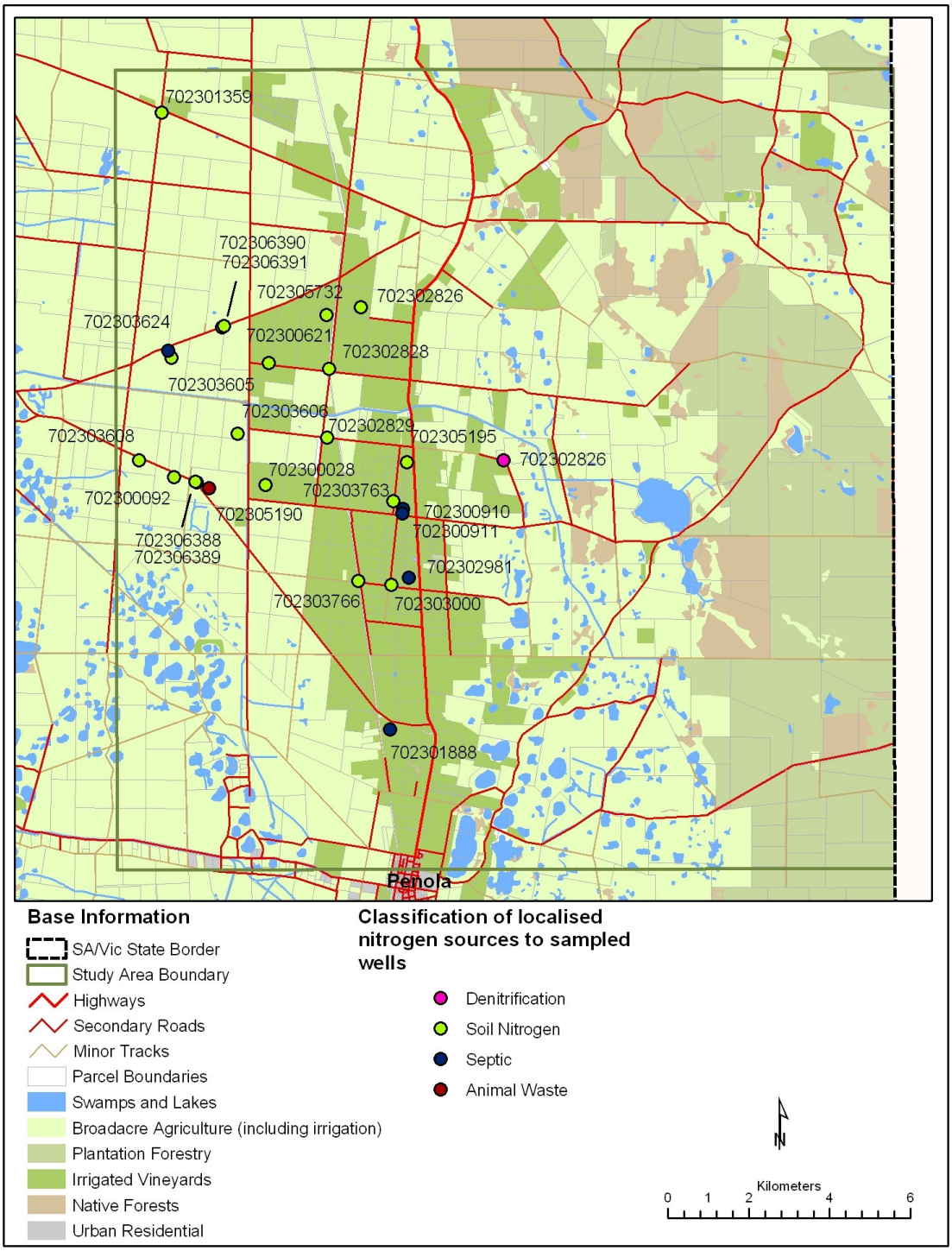

Figure 6.6: The categorisation of wells sampled for nitrate isotopes

Only one well was close to a water trough and so expected to be impacted by animal waste, and this was not differentiated from the septic waste (by nitrogen isotope data).

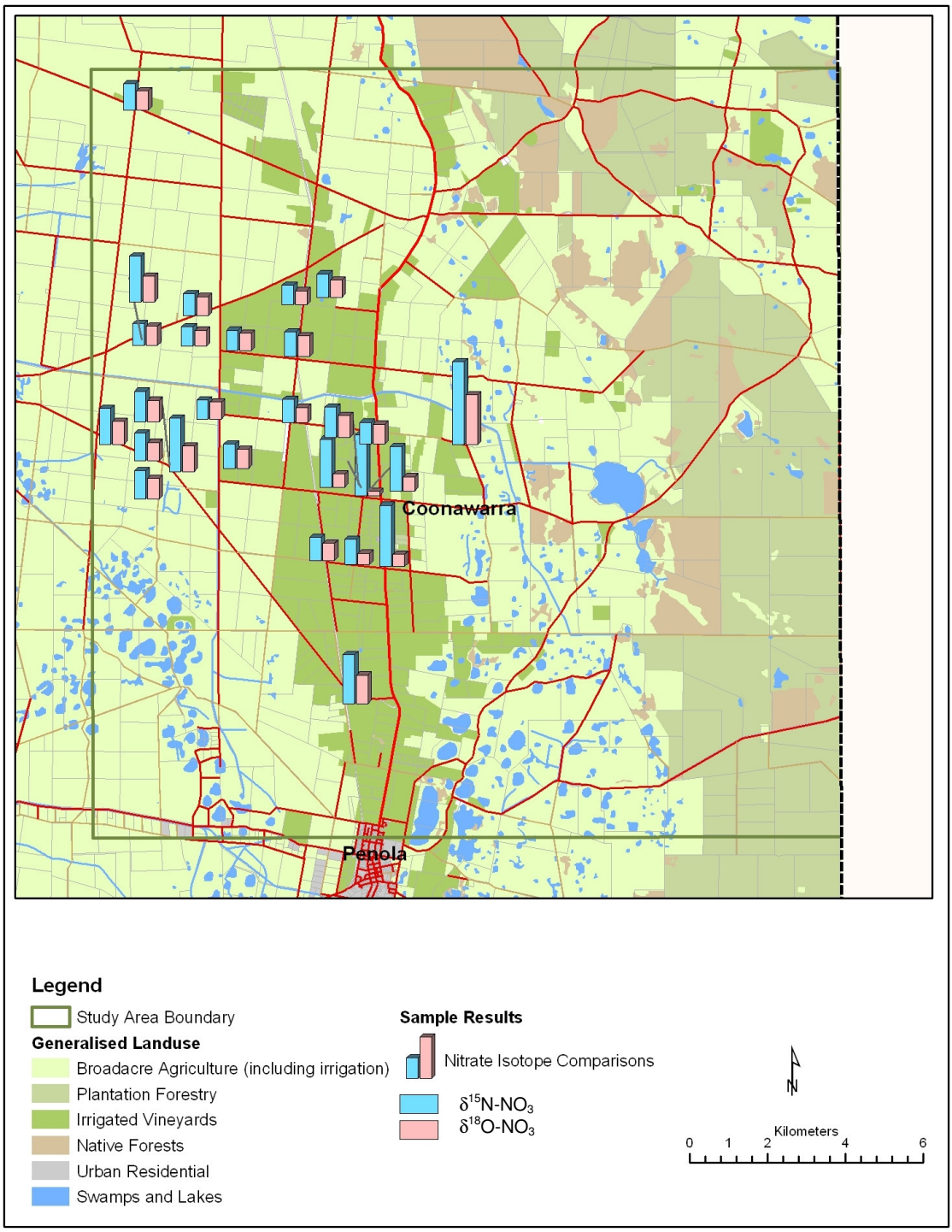

Figure 6.7: **The nitrogen and oxygen isotope comparisons of groundwater within the study area**

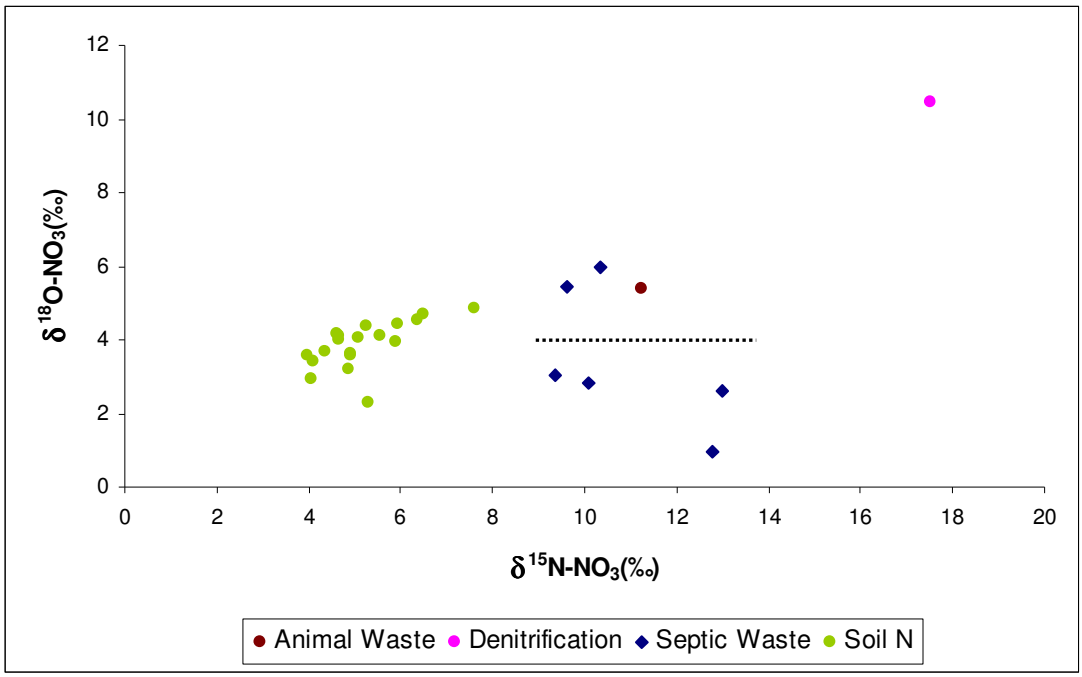

Figure 6.8: Categorised nitrate isotope comparisons in groundwater within the study area (see text for dotted line)

The single outlier (in Figure 6.8) identified as demonstrating denitrification is discussed below.

The isotopic composition of the nitrate proposed to have been generated from soil nitrification has a relatively small range ($\delta^{15}N$ of +4.0‰ to +7.6‰, and $\delta^{18}O$ of +2.3‰ to +4.9‰), compared to the wider range proposed to have originated from septic waste ($\delta^{15}N$ of +9.4‰ to +13.0‰, and $\delta^{18}O$ of +0.9‰ to +6.0‰), with the range of $\delta^{18}O$ of the nitrate being approximately half the range in the septic waste sourced nitrate. The wells with the proposed nitrate origin of septic waste are also spatially distinct (Figure 6.6).

The dotted line in Figure 6.8 differentiates those wells that are within the central Coonawarra township (below the dotted line) and those wells significantly separated from the township.

There are two potential causes for this difference (although the additional reason of coincidence cannot be entirely excluded given the small sample sizes). One reason is that the nitrate isotope ratio attributed to septic waste

sources away from the township of Coonawarra is not actually from septic waste, but is the result of the denitrification of nitrate produced by microbial soil nitrification. Another potential cause is mixing of groundwater from two or more sources. Both of these issues are discussed in the next section.

Another potential cause that was considered but discounted was that the oxygen for nitrification is sourced differently (and therefore has different isotopic composition) in the two areas. The reason for discounting this cause is that there is no justification for a difference in the atmospheric O_2 isotopic composition within the study site, and the only two sources of $\delta^{18}O$ of the water (used for toilet flushing) within the study area have the same isotopic composition; groundwater has a $\delta^{18}O$ of approximately -4.5‰ (Love, et al. 1992), and rainwater has a $\delta^{18}O$ of approximately -3.5‰ to -4.4‰ (based upon rainfall data between 1990 and 2000 for Adelaide and Melbourne);(IAEA/WMO 2004).

The comparison of nitrate concentrations and $\delta^{15}N$ in groundwater (Figure 6.9) further shows that the septic waste sources have resulted in ratios that are significantly different from microbial soil nitrification. As for the distribution in Figure 6.8, the wells suspected to have been impacted by septic waste in the Coonawarra township are differentiated from those wells outside of the township also suspected to be impacted by septic waste (those to the right of the dotted line in Figure 6.9 are within the central Coonawarra township). In this case the differentiation is based upon nitrate concentrations.

A single well result is tentatively attributed to animal waste. This well is a stock watering point, and while spatially located within a cropping area, is expected to be dominated by the presence of animal waste. The well is grouped (in Figures 6.8 and 6.9) with those wells located near septic systems, however there are no septic systems near this well. The well displays relatively high $\delta^{18}O$ and $\delta^{15}N$ for nitrate, as well as a relatively high nitrate concentration. While mixing and/or denitrification are not discounted (see discussion below), the nitrate isotope composition (and the nitrate

concentration, and the field assessment of landuse) provides a strong

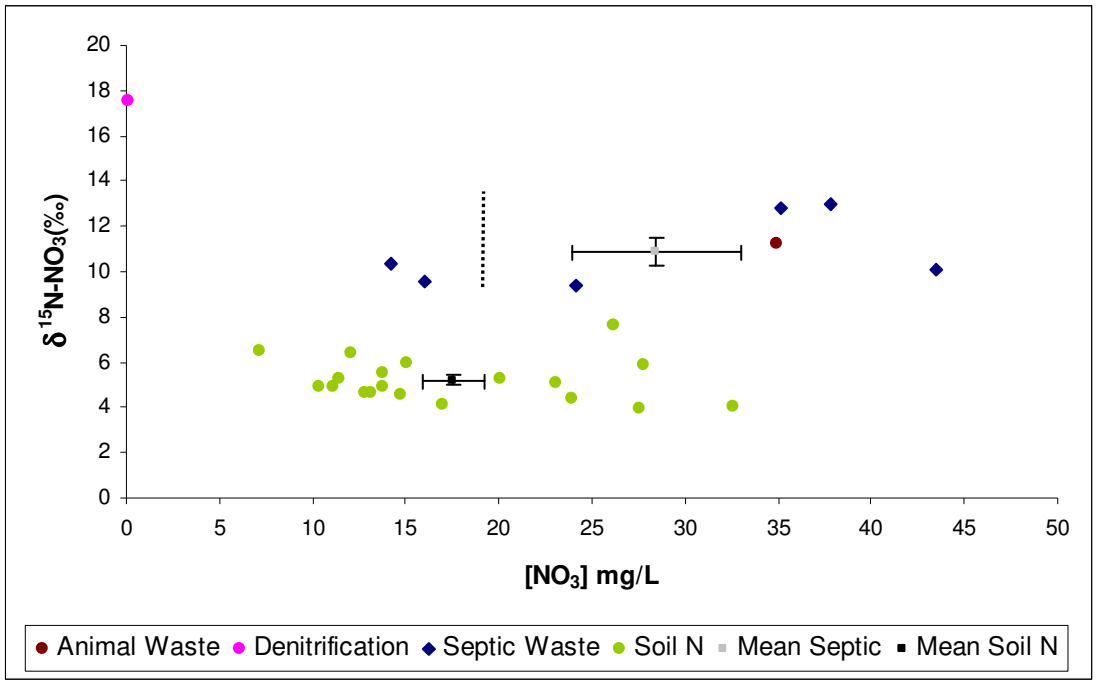

Figure 6.9: Nitrate concentrations and $\delta^{15}N$ of nitrate categorised within the study area (means and standard errors of septic waste impacted wells and soil microbial nitrification sources included). Significance of the dotted line is discussed in the text

indication that the nitrate source for this well is animal waste associated with livestock congregating at a nearby watering point.

6.3.2 Denitrification and Mixing

A variety of studies have been able to demonstrate that the combined isotopic composition of nitrate is able to identify the occurrence of denitrification within groundwater systems.

As presented in Figure 6.5, the occurrence of denitrification in the aquifer can be indicated through the predicted proportional increase in the $\delta^{15}N$:$\delta^{18}O$ values at a ratio of approximately 1.96:1, and the plotted groundwater data

for all of the wells generally reflect this tendency (Figure 6.8). The single well labelled as 'denitrification' in Figure 6.6 is located in the north east of the study area and has high $\delta^{15}N$ and $\delta^{18}O$ values, coupled with the very low nitrate concentration. Unfortunately, it is not possible to accurately determine the source of this nitrate, however given the sites location, it is more probable to be either generated from soil microbial nitrification or animal waste (an abandoned dairy is located approximately 100 metres up gradient of this well).

As discussed above, the conclusions that can be drawn from the well that is predicted to have been impacted by animal waste (well 702305190) must be considered cautiously. However, the isotopic composition of the nitrate is in the lower range (for both $\delta^{15}N$ and $\delta^{18}O$) reported in the literature for animal waste, and therefore it is proposed that while denitrification (and mixing) cannot be discounted, these mechanisms are not likely to have been significant at this well. The nitrate concentration at this well is high (approximately 35 mg/L), further supporting this view.

Those wells that are identified as being likely to have been impacted by soil microbial nitrification present ratios that are aligned to the theoretical denitrification ratio, indicating that denitrification may be occurring in groundwater across the study area. A further assessment showed that there was no spatial relationship within this perceived trend. Using theoretical estimations, the degree of denitrification is further investigated for these wells.

Application of the isotopic approach is possible because of the fractionation that occurs during the anaerobic denitrification process. As denitrification occurs, the isotopic ratio of the remaining nitrate reactant will increase exponentially as its concentration decreases. The Rayleigh equation (equation 6.6) describes the isotopic composition of the denitrification product (N_2) and the residual nitrate substrate;

$$\delta_t = \delta_0 + \varepsilon.\ln\frac{[NO_3^-]_t}{[NO_3^-]_0}$$ Equation 6.6

where δ_t is the isotopic composition at time *t*, δ_0 is the original isotopic composition, ε is the isotopic fractionation factor, $[NO_3^-]_0$ is the original nitrate concentration, and $[NO_3^-]_t$ is the nitrate concentration at time *t* (Chen and MacQuarrie 2005).

Using this relationship, Mariotti and his colleagues (1988) proposed that it could be determined whether nitrate in groundwater was the subject of denitrification or primary mixing. If denitrification is most important there should be a direct relationship between the $\delta^{15}N$ and the natural log of the nitrate concentration. This test was applied to the nitrogen isotope data but it did not produce a significant correlation to suggest that denitrification was the dominant process occurring across all of the sampled wells.

The main difficultly with applying this method is that the original source of nitrate (and therefore the nitrogen and oxygen isotopes ratios) is not confirmed. One exception is the well classified as denitrification in Figure 6.8. Evidence for denitrification here is a high nitrogen and oxygen isotope values together with a low nitrate concentration.

The last group of wells within the study area are those that are suspected to have been impacted by septic waste. The variability observed in these wells has been noted above, particularly that wells within Coonawarra display a reduced $\delta^{18}O$, and a higher nitrate concentration compared to those wells outside of the township (Figures 6.7 and 6.8 respectively).

There are a limited number of wells of this type, and therefore quantitative analysis of trends is not appropriate. However a qualitative assessment suggests that there is no substantial evidence of denitrification occurring within the aquifer (see above discussion), or that denitrification is responsible for the variability observed within this category. The significantly lower nitrate concentrations away from the township of Coonawarra (for this well class)

without the change in nitrogen isotope composition suggests dilution of the originating septic water with water of a lower nitrate concentration; such an observation was made by Mariotti and his colleagues (1988). While the majority of the study area has elevated concentrations of nitrate, the sampling of groundwater has indicated that there are areas where there is essentially nitrate-free water (see Table 6.1), and therefore dilution may be more dominant in these areas.

6.4. DISCUSSION

The investigation of the $\delta^{15}N$ and $\delta^{18}O$ composition of nitrate within the study area provides strong evidence that the nitrate within the groundwater originates from at least two different sources; septic waste and soil microbial nitrification (of organic nitrogen). The possible direct infiltration of nitrate from fertiliser application is not supported by the results, and neither are atmospheric sources of nitrate. Potential nitrification of commercial fertilisers could contribute to the elevated nitrate concentrations. However if significant it should result in higher $\delta^{18}O$ values for nitrate than were observed in this study (Hübner 1986).

Winery wastewater's nitrate contribution to groundwater was not able to be resolved using this approach, primarily because the isotopic composition of the wastewater was not determined. Literature values for the expected isotopic composition of winery wastewater were not available. Thus, although wells within Coonawarra township are suggested to have been impacted primarily by septic waste originated nitrate, the contribution of nitrate from winery wastewater cannot be discounted. However, as discussed in Chapter 5, the quantities and concentration of nitrate within winery wastewater are unlikely to have resulted in the widespread contamination of the aquifer (based on a mass-balance).

The suggestion that septic waste is likely to be responsible for elevated concentrations of nitrate into groundwater around Coonawarra is supported by the nitrate isotopic composition, and field observations of the close

proximity of septic tanks and extraction wells. This is supported by numerous studies that show septic tanks result in these types of impacts (Hantzsche and Finnemore 1992, Hallberg and Keeney 1993).

This conclusion further supports the earlier observations in this thesis (Chapter 2) that the use of these wells to develop regional groundwater quality maps (such as through kriging algorithms) is flawed and will result in an over-estimation of nitrate concentrations.

This research has also indicated that the main source of nitrate to groundwater across the wider study area is from the nitrification of soil nitrogen (primarily organic nitrogen although commercial fertiliser cannot be discounted). Other studies within the region have reported considerable nitrate leaching to groundwater from soil organic nitrogen as a result of cultivation techniques (Dillon, et al. 2000), and this has been described in numerous texts as a potentially significant mechanism for nitrate entering groundwater systems (Lawrence 1983, Keeney 1986, Knight and Tuckwell 1988, Keeney 1989, Thomas, et al. 1989, Worrall and Burt 1999, 2001).

The western part of the study area is subject to regular cultivation, and leguminous crops (primarily Faba Beans) are a commonly grown crop in the area. Leguminous crops have previously been proposed as a contributing source of nitrate to the regional unconfined aquifer (Schmidt, et al. 1998), and a combination of cultivation and this cropping type is consistent with the isotopic and concentration data collected for nitrate in groundwater. This may therefore present a significant source of nitrate to the aquifer in this location.

Nitrate concentrations under the wider Coonawarra vineyard area are also elevated, and have isotopic compositions that strongly indicate soil organic nitrogen is the nitrate source. As explained in Chapter 1, while some inter-row spaces in vineyards are cultivated, most are simply mown or occasionally sprayed (with herbicide). Once established these vineyards receive very little nitrogen based fertiliser. It is proposed that the elevated

nitrate in these areas is primarily caused by increased percolation resulting from irrigation, and/or the shallow nature of the soil. This is particularly the case with frost-irrigation applied during the winter, when large volumes are applied to prevent frost damage to vines.

A dual isotopic composition approach was used to determine the extent of denitrification within the study area aquifer. Results from one well in the central north-eastern part of the study area provides evidence of denitrification, however the degree of denitrification could not be calculated due to uncertainty about the nitrate source composition. Data from the remaining wells was unable to demonstrate that denitrification was occurring across the wider study area, primarily because there was little variability within the nitrate isotopic composition from the wells impacted by different sources. This result suggests that the spatial trend in nitrate concentrations (a west to east reduction in groundwater nitrate) is not explained by in-aquifer denitrification.

6.5. CONCLUSION

Use of the isotopic composition of nitrate for source determination has provided evidence of two main sources of nitrate to the area's groundwater; septic waste and soil organic nitrate. Nitrogen isotopic methods have not previously been used within the South East region for source determination, and the dual isotopic method has not previously been reported for Australian groundwater nitrate studies.

The method provides additional confirmation that diffuse sources are a major contributor to elevated nitrate concentrations in groundwater. This supports the earlier findings of Waterhouse (1977), Harvey (1979), Schmidt et al. (1998), Dillon (1988), and Lawrence (1983).

Results suggest that there is a significant localised impact from the operation of septic tanks within the Coonawarra area, and that these sources dominate other diffuse sources. Many other studies have highlighted that nitrate

concentrations in groundwater are likely to be the result of localised impacts. The research also provides further confirmation that the use of point-source nitrate groundwater data to create groundwater quality maps may be flawed and can result in over-estimation of groundwater contamination levels if wells are poorly located.

Finally, the application of this method has demonstrated that in areas within the study area, the aquifer does not appear to be well mixed. Between some wells within close proximity it was observed that there were significant differences in concentration and isotopic composition. This further suggests that well selection for ambient groundwater assessments need to consider localised sources to avoid prejudicing monitoring results.