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HYDROLOGICAL VARIABILITY, ORGANIC MATTER SUPPLY AND DENITRIFICATION IN THE GARONNE RIVER ECOSYSTEM

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RUNNING HEAD: Garonne River Denitrification

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SUMMARY

1. Groundwater nitrate contamination has become a worldwide problem as increasing amounts of nitrogen fertilizers are used in agriculture. Alluvial groundwater is uniquely juxtaposed between soils and streams. Hydrological connections among these subsystems regulate nutrient cycling.

2. We measured denitrification using an *in-situ* acetylene-block assay in a nitrate contaminated portion of the Garonne River catchment along a gradient of surface water-ground water mixing during high (snowmelt) and low flow.

3. During high flow (mid-April to early June) the water table rose an average of 35 cm and river water penetrated the subsurface to a great extent in monitoring wells. Denitrification rates averaged 5.40 $\mu g N_2O\ L^{-1}\ min^{-1}$ during the high flow period, nearly double the average rate (2.91 $\mu g N_2O\ L^{-1}\ min^{-1}$) measured during base flow. This was driven by a strong increase in denitrification in groundwater under native riparian vegetation. Nitrate concentrations were significantly lower during high flow compared to base flow. Riparian patches had higher dissolved organic carbon (DOC) concentrations that were more aromatic compared to the gravel bar patch closest to the river.

4. Multiple linear regression showed that the rate of denitrification was best predicted by the concentration of low molecular weight organic acids. These molecules are probably derived from decomposition of soil organic matter and

are an important energy source for anaerobic respiratory processes like denitrification. The second best predictor was per cent surface water, reflecting higher denitrification rates during spring when hydrologic connection between surface water and ground water was greatest.

5. Our results indicate that, while denitrification rates in Garonne River alluvium were spatially and temporally variable, denitrification was a significant NO_3 sink during transport from the NO_3-contaminated floodplain to the river. DOC availability and river-floodplain connectivity were important factors influencing observed spatial and temporal patterns.

INTRODUCTION

The role of hydrological linkages among subsystems within catchments has received increased attention in recent years by stream ecologists and hydrologists. The groundwater/surface water interface is recognized as an important hotspot of nutrient retention and transformation, a habitat for a unique and diverse invertebrate community, and a significant contributor to ecosystem resistance and resilience to disturbance (see reviews in Jones & Holmes, 1996; Brunke & Gonser, 1997; Boulton *et al.*, 1998; Dahm *et al.*, 1998). The region of seasonal saturation, or that portion of the soil column that is seasonally saturated by a rising groundwater table, is recognized by wetland ecologists as an important source of water and nutrients to floods at the land surface (Bartley & Croome, 1999). This interface has received little attention by stream and groundwater ecologists, although it can supply dissolved organic matter and other solutes to both surface water and groundwater (Baker, Valett & Dahm, 2000). Denitrification is a microbially mediated process in anoxic sediments where nitrate nitrogen (NO_3-N) is converted to N_2 gas during the acquisition of energy. It may be heterotrophic, with organic substrates as the energy source, or autotrophic, with reduced inorganic compounds such as sulphur or iron used as electron donors (Korom 1992). The ecological significance of this process is twofold: first, denitrification is a permanent sink for nitrogen, which is a nutrient that can control aquatic primary production; second, denitrification is a process by which NO_3-N, a common groundwater pollutant that may impact human health *(e.g.*, van Egmond, Bresser & Bouwman, 2002; Wolfe & Patz, 2002), can be removed from water without chemical

treatment. Understanding the importance of denitrification to riverine N budgets and the factors that influence its rate are therefore important to basic and applied ecology.

Microbial heterotrophic activity in groundwater and the hyporheic zone has been shown to be limited by the availability of organic matter (OM; Jones, 1995; Baker, Valett & Dahm, 2000). Since denitrification can be a heterotrophic process, it is reasonable to expect that it will also be influenced by organic matter availability (*e.g.*, Hill *et al.*, 2000). Dissolved organic carbon (DOC) quality can also affect denitrification (Pfenning & McMahon, 1997). Both surface water and soils have been shown to be sources of organic matter for subsurface heterotrophic metabolism (*e.g.*, denitrification; Jones, 1995; Pfenning & McMahon, 1997; Baker, Valett & Dahm, 2000); however, the primary OM source probably depends on the magnitude and direction of interaction with catchment subsystems.

Studies of hydrological linkages among streams, soils and groundwater are mostly limited to lower order streams (but see Hinkle *et al.*, 2001). In larger rivers, the hydrologic regime influences the degree of interaction among soils, groundwater and surface water. At low flow, rivers are primarily influenced by groundwater discharge, and surface water penetration into the subsurface is limited. During high flow, river water penetrates far into the subsurface and the groundwater table rises, hydrologically linking soils to groundwater and surface water. These varying hydrologic conditions can alter substrate availability for microbial processes like heterotrophic dentirification. To understand its potential importance to the N budget, and its range of temporal variability, we measured denitrification during high flow and low flow along a gradient

of surface water - groundwater mixing in the nitrate contaminated alluvial aquifer of the Garonne River, France. We hypothesized that substrate availability (DOC and NO_3-N) limits denitrification, and that hydrological conditions (high vs. low flow) could alter substrate availability. Several factors may be working in concert to affect denitrification rates during the different flow conditions. These include:

1. During high flow, penetration of surface water into the subsurface may increase DO concentrations and inhibit denitrification.

2. During high flow, a rising water table may induce anoxia in the newly saturated substratum, facilitating denitrification.

3. During high flow, penetration of surface water may dilute subsurface NO_3-N concentration to sufficiently limit denitrification.

4. High flow can increase the amount of DOC in the subsurface, increasing heterotrophic denitrification rates compared to low flow conditions.

If conditions 1 and / or 3 above occurred, we expected to observe lower denitrification rates during snow melt. Scenarios 1 and 3 could be separated based on changes in dissolved oxygen (DO) and NO_3-N, respectively. In contrast, if scenario 2 and / or 4 regulate denitrification, we expected to measure higher denitrification rates during snow melt. Situation 2 could be separated from 4 if DO concentration was lower, and DOC concentration was higher, during snowmelt compared to low flow.

METHODS

Study site

We conducted our work along a 2-km meander of the Garonne River near the village of Monbéqui, 50km north of Toulouse, France (43º53'N 1º12'E) (Fig. 1). At this location, the river is seventh order with a mean annual discharge of 200 $m^3 s^{-1}$. The river flows over coarse glacial deposits that are 4-6 m deep. In this area, most of the riparian vegetation has been cleared and the floodplain is intensively farmed. Sites closest to the river are often managed as poplar (*Populus alba* L.) plantations with a maximum stand age of 20 years. Other main crops in the area include wheat (*Triticum* sp.), corn (*Zea mays* L.), and sunflower (*Helianthus annus* L.) (CNRS, 1991). Application of chemical fertilizers has resulted in groundwater nitrate contamination, with concentrations often exceeding 11 mgN L^{-1}, the drinking water standard set by the European Union (CNRS, 1991).

The Monbéqui study site has three types of vegetative cover: gravel bar with pioneer riparian vegetation *(Salix alba* L.*),* native riparian zone dominated by *Salix alba, Populus alba, P. nigra* L.*, and Fraxinus angustifola* Vahl with an understory of *Urtica dioica* Linne, and a poplar plantation / wheat field that experiences biannual tilling and occasional fertilization (Fig. 2). The area is not protected by dikes and the gravel bar portion floods annually, while the native riparian zone floods on average once every 7 years (Pinay *et al.,* 2000). The site was instrumented in 1992 with 30 piezometers constructed of either 2 cm (gravel bar) or 6.3 cm (riparian and poplar plantation) diameter polyvinyl chloride (PVC pipe with full screens that extended to underlying marl substratum (Fig. 2; Pinay *et al.,* 1998; Lambs, 2000; Pinay *et al.,* 2000).

Hydrodynamics and sample collections

Garonne River discharge was measured daily at a gauging station at Verdun sur Garonne, 3 km upstream of the study site. To determine the influence of flooding on groundwater denitrification rates and subsurface biogeochemistry, we selected three wells in each land cover type (one well in the poplar plantation was destroyed by a tractor and so was not included in further analyses). Sample times were chosen based on the hydrograph; spring flood sampling occurred on the rising limb of the hydrograph (mid-April until early May 1999) and summer sampling occurred on the descending limb of the hydrograph as the river returned to base flow (mid July 1999, Fig. 3). On each sample date we recorded water table height, electrical conductivity and temperature. Water samples were collected from each well using a peristaltic pump. To ensure sampling from the same depth in the subsurface, the pump was equipped with an inflatable bladder which sealed a 20-cm section of well screen (see Fig. 4). Sampling occurred 10 cm above the bottom of each well. Unfiltered water samples were pumped into 25 ml BOD bottles for DO analysis and directly into a 60 ml syringe for nitrous oxide (N_2O) gas collection. Nitrous oxide was extracted from the water by headspace equilibration (Weiss & Price, 1980) and collected into evacuated vials (Venoject, Leuven Belgium) for later analysis. Water samples were filtered using 47 mm Gelman in-line filter holders with ashed Whatman GF/F filters (0.7 μm pore size) into acid-rinsed high-density polyethylene (HDPE) bottles and stored on ice until return to the laboratory. Filtered water samples were also collected from the Garonne River at

the study site and from an agricultural well several kilometres away from the river.

Solute and dissolved gas analyses

DO was quantified using Winkler titrations (Wetzel & Likens, 1991). Dissolved N_2O was measured following the method of Weiss & Price (1980) on a gas chromatograph with a ^{63}Ni electron capture detector plumbed with back-flush to vent and Poropak Q columns (Girdel Model 30). Anions, including NO_3-N, chloride (Cl^-), bromide (Br^-), phosphate (PO_4-P), sulphate (SO_4-S) and low molecular weight organic acids (LMW-OA), including acetate, formate, lactate, butyrate and propionate were determined by ion chromatography using a DIONEX 500 with gradient pump, sodium hydroxide mobile phase, and AS11-HC analytical and guard column sets (Baker, Dahm & Valett, 1999). The LMW-OA concentrations were summed for total LMW-OA concentration and expressed as a percentage of total DOC concentration for subsequent data analyses. DOC was measured after UV oxidation on a Shimadzu 5000-A TOC analyser. Ultra violet absorbance was measured on acidified samples at 254 nm on a fixed wavelength spectrophotometer with 5 cm-path length quartz cuvettes and converted to absorbance per metre (Westerhoff & Anning, 2000). Specific ultra violet absorbance (SUVA), a measure of DOC aromaticity, was calculated as the ratio of absorbance to DOC concentration (Westerhoff & Anning, 2000).

End member mixing analysis

Mixing of surface and subsurface water masses was estimated for each well by a

two-end-member mixing analysis (EMMA) using Cl^- concentrations observed in river water and the agricultural well (Mulholland & Hill, 1997; Pinay *et al.*, 1998). EMMA solves the equations

$$[Cl^-_{well}] = [Cl^-_{SW}]f_{SW} + [Cl^-_{GW}]f_{GW}$$

$$1 = f_{SW} + f_{GW}$$

where $[Cl^-_{well}]$ is the estimated Cl^- concentration in the well, $[Cl^-_{SW}]$ is measured river water Cl^- concentration, $[Cl^-_{GW}]$ is Cl^- concentration in groundwater measured in the agricultural well and f_{SW} and f_{GW} are the fraction of water derived from surface and groundwater respectively. EMMA assumes that there are no precipitation inputs and that Cl^- is chemically and biologically conservative. These assumptions are reasonable given past hydrological research at the site which showed a nearly 1:1 relationship between electrical conductivity and $\delta\,^{18}O$ (Lambs, 2000). Measured river Cl^- concentrations were 7.1 mg L^{-1} and 11.9 mg L^{-1} for spring and summer, respectively. Agricultural well Cl^- concentrations were 79.0 mg L^{-1} during spring and 61.1 mg L^{-1} during summer. We calculated predicted NO_3-N concentration based on a similar EMMA, substituting observed surface water and agricultural well NO_3-N concentrations (1.03 and 16.9 mg NO_3-N L^{-1} during spring and 1.68 and 12.3 mg NO_3-N L^{-1} during summer) for Cl^-. This allowed for estimation of NO_3-N retention via uptake or denitrification compared to dilution by low-NO_3-N surface water.

Denitrification

Denitrification rates were measured using the push-pull, *in-situ* acetylene block technique (Istok *et al.*, 1997; Addy *et al.*, 2002; Sanchez-Perez *et al.*, 2003). After background water chemistry samples were collected for the analyses described above, 20 L of groundwater was pumped into a carboy and was mixed with KBr to bring the Br^- concentration to 10X background (5-8 mg Br L^{-1}) for use as a conservative water-mass tracer. Calcium carbide (ca. 25 g) was reacted with water in a 200 ml sealed glass serum bottle to bubble acetylene into the carboy for 5 minutes (Fig. 4). The acetylene-saturated water was then pumped back into the same well (push, Fig. 4). During the push phase, we continued bubbling the water in the carboy with acetylene. Water samples were collected immediately following injection and at 30 minute intervals for 2-4 hours for analysis of N_2O and anions as described above (pull). Denitrification rates were calculated as the rate of increase in dilution-corrected N_2O concentration (Fig. 5).

Statistics

Repeated measures analysis of variance (ANOVA) was used to test for spatial and temporal differences in dissolved solutes and denitrification rates (PROC GLM; SAS, 1997). Multiple linear regression was used to assess which hydrological and biogeochemical parameters best predicted measured denitrification rate. The inclusion criterion was set *a priori* at alpha = 0.15 (PROC REG; SAS, 1997) .

RESULTS

Hydrodynamics

Mean annual discharge in the Garonne River was 200 m^3s^{-1}. During the study there were several floods greater than 500 m^3s^{-1} (Fig. 3). Aside from these spates, the highest discharge occurred from mid-April until June 1999 (Fig. 3) due to snowmelt in the upper reaches of the catchment. Base flow (ca. 75 m^3s^{-1}) was reached in late July and extended through the end of the water year (Fig. 3). During spring high flow (April-June), water table height in the study piezometers was an average of 35 cm higher than the water table during base flow (July-October). Subsurface water table was 0.5-3 m below the ground surface during base flow.

Hydrologic conditions influenced river water penetration into the subsurface as determined by EMMA. Surface water represented the majority of water in wells during spring high flow, averaging 59 - 88% in the different well types (Table 1). The amount of surface water in wells was substantially lower during base flow. Wells contained 11-65% of surface water at that time (Table 1). Under both flow conditions, gravel bar wells contained the most surface water, while wells in the poplar plantation contained the least (Table 1).

Biogeochemistry

DO was present in all wells during both sample periods. During high flow, DO was 3.37$\pm$0.83 mg L^{-1}. DO was slightly lower during base flow, averaging 2.69$\pm$0.63 mg L^{-1}. Differences were not statistically significant among well types or flow conditions

(repeated measures ANOVA $p>0.05$).

Nitrate concentrations were significantly lower during high flow compared to low flow (repeated measures ANOVA $p=0.025$). Mean concentration ranged from 0.156 to 5.27 mg NO_3-N L^{-1} during high flow and 3.26 to 10.7 mg NO_3-N L^{-1} during baseflow (Table 1). NO_3-N concentration was not statistically different among well types; however, gravel bar wells consistently exhibited the lowest concentration and poplar plantation wells had the highest concentration (Table 1). Observed NO_3-N concentration was 96-42% lower than predicted by EMMA during high flow (Table 1; Fig. 6). During baseflow, mixing accounted for a greater proportion of NO_3-N loss, as the observed concentration was only 6-50% lower than predicted by EMMA (Table 1; Fig. 6). In two wells during summer, measured NO_3-N concentration was higher than predicted from mixing, indicating NO_3-N production (Fig. 6).

Organic matter content (DOC) and composition (SUVA and LMW-OA), were variable and exhibited no statistically significant pattern with flow regime (Table 2). DOC averaged 2.2 and 2.4 mg C L^{-1} across well types during high and low flow, respectively (Table 2). SUVA ranged from 0.1 to 1.1 m^{-1} (mg $L^{-1})^{-1}$ and was highest under the poplar plantation and native riparian vegetation during high and low flow, respectively (Table 2). The proportion of DOC that was represented by Low molecular weight organic acids, namely acetate, formate, lactate and butyrate (propionate was not detected in any sample), did not exhibit spatial or temporal patterns and ranged from 1 to 9 % of total DOC (Table 2).

Denitrification

The *in-situ* acetylene block assay resulted in increased dissolved N_2O concentration. In most cases, gas production was concomitant with the decrease in NO_3-N concentration (Fig. 5), and no change in Cl^- or SO_4-S. Heterotrophic denitrification was indicated by the stoichiometric ratio of N_2O-N production:NO_3-N consumption, which averaged 1.15. Denitrification rates were highly variable among well types and seasons (Fig. 7). Overall, denitrification nearly doubled during the spring high flow period, averaging 5.40 μg N_2O produced L^{-1} min^{-1} during spring flood compared to 2.91 μg N_2O produced L^{-1} min^{-1} during summer base flow. This was driven by a large increase in denitrification in native riparian patches. Denitrification rates were significantly higher during spring in groundwater under native riparian vegetation than in either the near-river gravel bar or the poplar plantation / wheat field (repeated measures ANOVA $p= 0.012$; Fig. 7). Interaction between time and location was not statistically significant (repeated measures ANOVA $p=0.07$).

Denitrification was not significantly correlated with DOC concentration. Multiple linear regression showed that the best predictor of denitrification rate was total Low molecular weight organic acids amount, which accounted for 40% of the variation in denitrification rate ($p<0.10$). Percent surface water was the second-best predictor of denitrification, and accounted for an additional 12% of variation in denitrification rate.

DISCUSSION

Our results confirm the presence of significant surface water-groundwater exchange in the alluvium near the Garonne River. Pinay *et al.* (1998) identified a similar pattern at this study site. We further showed that the interaction between surface and groundwater changed temporally, with greater surface water penetration during spring high flow.

Hydrological changes during high flow had important biogeochemical consequences. First, NO_3-N concentration in groundwater was significantly reduced. This was also documented in the study by Pinay *et al.* (1998), in which they attributed a large portion of the seasonal NO_3-N decrease to dilution by NO_3-poor river water. Our results show that this is not necessarily the case, especially during high flow. Although wells contained a greater amount of surface water during high flow, observed NO_3-N concentrations were 42-96% lower than those predicted from dilution alone. Indeed, biologically mediated loss must not be discounted. The discrepancy between the two data sets may result from differences in the exact location of measurement, as subsurface biogeochemistry is spatially variable, and / or from hydrological differences between the years of study.

Pinay *et al.* (1998) argued that groundwater denitrification at Monbéqui should be minimal due to the presence of dissolved oxygen. In our study, dissolved oxygen concentration in the wells exceeded 2 mg L^{-1} on average. Despite this, we measured substantial rates of denitrification. We estimated NO_3-N loss via denitrification during

transport by multiplying the daily rate and average groundwater advective velocity (Bats-Landalle 1998). Between 0.3-3.0 mgN L^{-1} m^{-1} was denitrified, indicating that the observed rates could account for all of the nitrate loss not due to mixing.

The rates of denitrification we measured in our study are comparable with those determined for coastal riparian areas in Rhode Island, USA using a similar method (Addy *et al.*, 2002). Our measured rates are considerably higher than those published for soils from Monbéqui (Pinay *et al.*, 1998; 2000). The *in-situ* push-pull technique allowed sampling deep in the aquifer without disrupting sediment microsites. Although few studies have used *in-situ* techniques to measure subsurface denitrification, the data that do exist show that denitrification rates measured *in-situ* are consistently higher than those measured using cores, chambers or mesocosms (Addy *et al.*, 2002).

The use of the acetlyene block technique to measure denitrification has fallen out of favour in recent years as acetylene also blocks nitrification (NH_4–> NO_3). The technique therefore underestimates denitrification in systems with low NO_3 concentrations that rely on nitrification as a NO_3 source (Blackmer *et al.*, 1980). Given that the Garonne system has high NO_3 concentrations relative to other fluvial ecosystems and to drinking water standards, our data represent reasonable estimates of denitrification at this site.

Higher denitrification rates observed in riparian wells at high flow were not induced by greater anoxia, as DO concentration exhibited no significant change between high and low flow. In fact, the average DO concentration measured during

high flow was slightly higher than that measured at low flow. Although DOC quantity and quality also did not change between high and low flow, multiple linear regression revealed that low molecular weight organic acid content and percent surface water were important predictors of denitrification rate. This suggests that DOC availability, specifically the labile low molecular weight organic acid fraction, and hydrology play important roles in regulating denitrification at this site. While DOC quantity alone was not a good predictor of denitrification rate, this could be due to increased substrate supply being balanced by an increased demand (*e.g.*, Dodds, 2003), resulting in no net change in organic substrate availability between flow periods.

The presence of low molecular weight organic acids in groundwater in the order of several percent of total DOC indicates substantial substrate available for heterotrophic denitrification. These molecules are fermentation products, the availability of which often limits anaerobic metabolism (Chapelle & Bradley, 1996). Waters of the Garonne River and its hyporheic zone also contain trace amounts of bioavailable saccharides, which likely fuel subsurface denitrification at this site (Vervier *et al.*, 2002).

Denitrification may be facilitated by riparian vegetation, which supplies organic matter to the subsurface via root production and litter fall (Schade *et al.*, 2001). Haycock & Pinay (1993) argue that flooding of the rooting zone can supply organic matter to the subsurface; however, most research designed to test this idea has focused on upper horizon soils (*e.g.*, Pinay *et al.*, 1998; Burt *et al.*, 1999; Clément, Pinay & Marmonier, 2002). Indeed, these studies show high denitrification potential in surface soils using

static cores or chambers, but do not address denitrification in riparian groundwater itself. Our observation of the highest rate of denitrification in groundwater under intact riparian vegetation with a well developed organic soil horizon is consistent with the hypothesis that soil-derived organic matter can fuel groundwater microbes.

Our data do not support conclusively the hypothesis that groundwater denitrification is limited by organic substrate availability and that this substrate can be supplied by river-floodplain interactions. Overall, we observed highest denitrification rates during high flow, when the river, groundwater and floodplain soils were most strongly connected. Low molecular weight organic acids content and percent surface water were important predictors of denitrification. Some combination of riverine C and soil C probably fuels denitrification. Finally, denitrification rates were spatially variable and the highest rates were observed during high flow in native riparian habitats compared to gravel bar and poplar plantation habitats.

In conclusion, the riparian areas of large river floodplains serve as important nutrient filters in catchments (*e.g.*, Peterjohn & Correll, 1984; Lowrance *et al.*, 1985; Pinay & Décamps, 1988). We have shown that, in addition to mixing between surface and subsurface water, NO_3-N losses in riparian groundwater can be accounted for by high rates of denitrification, and these rates are affected by hydrology and substrate availability. Catchment management practices that alter flooding regimes therefore have the potential to impact negatively the important ecosystem services provided by riparian zones.

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REFERENCES

Addy K., Kellogg D.Q., Gold A.J., Groffman P.M., Ferendo G. & Sawyer C. (2002) In-situ push-pull method to determine ground water denitrification in riparian zones. *Journal of Environmental Quality*, 31, 1017-1024.

American Public Health Association (APHA) (1998) *Standard Methods for the Examination of Water and Wastewater*, APHA.

Baker M.A., Dahm C.N. & Valett H.M. (1999) Acetate retention and metabolism in the hyporheic zone of a mountain stream. *Limnology and Oceanography*, **44**, 1530-1539.

Baker M.A., Valett H.M. & Dahm C.N. (2000) Organic carbon supply and metabolism in a near-stream groundwater ecosystem. *Ecology*, **81**, 3133-3148.

Bartley R.G. & Croome R. (1999) Groundwater influence on the balance and nutrient budget of a small natural wetland in northeastern Victoria, Australia. *Ecological Engineering*, **12**, 133-147.

Bats-Landalle G. (1998) *Analyse Géomorphologique et Hydrologique de la Plaine d'Inondation de la Garonne. Application à la Modélisation des Flux de Nitrates à l'Interface Nappe/Rivière*. DEA, Université Paul Sabatier, Toulouse.

Blackmer A.M., Bremner J.M. & Schmidt E.L. (1980) Production of nitrous oxide by ammonia-oxidizing chemoautotrophic microorganisms in soil. *Applied and Environmental Microbiology*, **40**, 1060-1066.

Boulton A.J., Findlay S., Marmonier P., Stanley E.H. & Valett H.M. (1998) The functional significance of the hyporheic zone. *Annual Review of Ecology and Systematics*, **29**, 59-81.

Brunke M. & Gonser T. (1997) The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biology*, **377**, 1-33.

Burt T.P., Matchett L.S., Goulding K.W.T., Webster C.P. & Haycock N.E. (1999) Denitrification in riparian buffer zones: the role of floodplain hydrology. *Hydrological Processes*, 13, 1451-1463.

Centre National de la Recherche Scientifique (CNRS) (1991) Les nitrates dans les vallées fluviales. *Programme Interdisciplinaire de Recherches Environnement*, Ministère de l'Environnement.

Chapelle F.H. & Bradley P.M. (1996) Microbial acetogenesis as a source of organic acids in ancient Atlantic Coastal Plain sediments. *Geology*, **24**, 925-928.

Clément J.-C., Pinay G. & Marmonier P. (2002) Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands. *Journal of Environmental Quality*, **31**, 1025-1037.

Dahm C.N., Baker M.A., Moore D.I. & Thibault J.R. (2003) Biogeochemistry of surface waters and alluvial ground waters in streams and rivers during drought. *Freshwater Biology*, In press.

Dahm C.N., Grimm N.B., Marmonier P., Valett H.M. & Vervier P. (1998) Nutrient dynamics at the interface between surface waters and groundwaters. *Freshwater Biology*, **40**, 427-451.

Haycock N.E. & Pinay G. (1993) Nitrate retention in grass and poplar vegetatedriparian buffer strips during the winter. *Journal of Environmental Quality*, **22**, 273-278.

Hill A.R., Devito K.J., Campagnolo S. & Sanmugadas K. (2000) Subsurface denitrification in a forest riparian zone: interactions between hydrology and supplies of nitrate and organic carbon. *Biogeochemistry*, **51**, 193-223.

Hill A.R. (1996) Nitrate removal in stream riparian zones. *Journal of Environmental Quality*, **25**, 743-755.

Hinkle S.R., Duff J.H., Triska F.J., Laenen A., Gates E.B., Bencala K.E., Wentz D.A. & Silva S.R. (2001) Linking hyporheic flow and nitrogen cycling near the Willamette River - a large river in Oregon USA. *Journal of Hydrology*, **244**, 157-180.

Istok J.D., Humphrey M.D., Schroth M.H., Hyman M.R. & O'Reilly K.T. (1997) Single-well, "push-pull" test for in-situ determination of microbial activities. *Ground Water*, **35**, 619-631.

Jones J.B. Jr. (1995) Factors controlling hyporheic respiration in a desert stream. *Freshwater Biology*, **34**, 91-99.

Jones J.B. Jr. & Holmes R.M. (1996) Surface-subsurface interactions in stream ecosystems. *Trends in Ecology and Evolution*, **11**, 239-242.

Lambs L. (2000) Correlation of conductivity and stable isotope 18O for the assessment of water origin in river system. *Chemical Geology*, **164**, 161-170.

Lowrance R.R., Todd R.L., Fail J. Jr., Hendrickson O. Jr., Leonard R. & Asmussen L. (1984) Riparian forests as nutrient filters in agricultural watersheds. *BioScience*, **34**, 374-377.

Mulholland P.J. & Hill W.R. (1997) Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: separating catchment flow path and in-stream effects. *Water Resources Research*, **33**, 1297-1306.

Peterjohn W.T. & Correll D.L. (1984) Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. *Ecology*, **65**, 1466-1475.

Pfenning K.S. & McMahon P.B. (1997) Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *Journal of Hydrology*, **187**, 283-295.

Pinay G., Black V.J., Planty-Tabacchi A.M., Gumiero B. & Décamps H. (2000)

Geomorphic control of denitrification in large river floodplain soils. *Biogeochemistry*, **30**, 9-29.

Pinay G. & Décamps H. (1988) The role of the riparian wood in regulating nitrogen fluxes between the alluvial aquifer and surface water: a conceptual model. *Regulated Rivers*, **2**, 507-516.

Pinay G., Ruffinoni C., Wondzell S. & Gazelle F. (1998) Change in groundwater nitrate concentration in a large river floodplain: Denitrification, uptake, or mixing? *Journal of the North American Benthological Society*, **17**, 179-189.

SAS Institute. (1999) *SAS Users Guide: Statistics*. SAS Institute.

Sanchez-Perez J.-M., Bouey C., Sauvage S., Teissier S., Antiguedad I. & Vervier P. (2003) A standardised method for measuring *in situ* denitrification in shallow aquifers: numerical validation and measurement in riparian wetlands. *Hydrology and Earth System Sciences*, **7,** 87-96.

Schade J.D., Fisher S.G., Grimm N.B. & Seddon J.A. (2001) The influence of a riparian shrub on nitrogen cycling in a Sonoran Desert stream. *Ecology*, **82**, 3363-3376.

Storey R.G., Fulthorpe R.R. & Williams D.D. (1999) Perspectives and predictions on the

microbial ecology of the hyporheic zone. *Freshwater Biology*, **41**, 119-130.

Tabacchi E., Lambs L., Guilloy H., Planty-Tabacchi A.M., Muller E. & Décamps H. (2000) Impacts of riparian vegetation of hydrological processes. *Hydrological Processes*, **14**, 2959-2976.

van Egmond K., Bresser T. & Bouwman L. (2002) The European nitrogen case. Ambio, **31**, 72-78.

Vervier P., Roques L., Baker M.A., Garabetian F. & Auriol P. (2002) Biodegredation of dissolved free simple carbohydrates in surface, hyporheic and riparian waters of a large river. *Archiv fur Bydrobiologie*, **15**, 595-604.

Weiss, R.F. & Price, B.A. (1980) Nitrous oxide solubility in water and seawater. Marine Chemistry, 8:347.

Westerhoff, P. & Anning D. (2000) Concentrations and characteristics of organic carbon in surface water in Arizona: influence of urbanization. *Journal of Hydrology*, **236**, 202-222.

Wetzel R.G. & Likens G.E. (1991) *Limnological Analyses*, Springer Verlag, NY.

Wolfe A.H. & Patz J.A. (2002) Reactive nitrogen and human health: acute and long-term implications. *Ambio,* **31**, 120-125.

Table 1. Subsurface hydrological and biogeochemical characteristics in gravel bar, native riparian and poplar plantation habitats along the Garonne during spring high flow and summer base flow, based on end member mixing analysis (EMMA). Data presented are means ± SE for each habitat type, significantly different means (repeated ANOVA p=0.025) are indicated by different superscripts.

	Observed Cl^- (mg L^{-1})	Observed NO_3-N (mg L^{-1})	% Surface Water	Predicted NO_3-N (mg L^{-1})	% NO_3-N Retention
High Flow					
Gravel Bar	16.3 ± 2.6	0.156 ± 0.13[a]	87.9 ± 3.6	2.96 ± 0.57	95.9 ± 3.1
Native Riparian	29.8 ± 4.6	2.54 ± 0.61[a]	69.1 ± 6.4	5.95 ± 1.0	58.3 ± 3.4
Poplar Plantation	37.1 ± 13	5.27 ± 4.0[a]	58.7 ± 19	7.58 ± 3.0	42.4 ± 30
Baseflow					
Gravel Bar	29.3 ± 7.7	3.26 ± 2.0[b]	64.6 ± 16	5.63 ± 1.7	51.7 ± 16
Native Riparian	46.1 ± 17	9.23 ± 4.7[b]	32 ± 32	9.25 ± 3.6	5.80 ± 15
Poplar Plantation	55.7 ± 4.5	10.7 ± 2.5[b]	11.4 ± 10	11.6 ± 1.0	9.00 ± 14

Table 2. Amount and characteristics of dissolved organic carbon (DOC) in gravel bar, native riparian and poplar plantation habitats along the Garonne River during spring high flow and summer base flow. Data presented are means ± SE for each habitat type. Within a column, significantly different means (repeated ANOVA $p<0.025$) are indicated by different superscripts.

	DOC (mg L^{-1})	SUVA (abs mg L^{-1} m^{-1})	Organic Acid Carbon Concentration (mg L^{-1})	% LMW OA
High Flow				
Gravel Bar	1.85 ± 0.17^{a}	0.251 ± 0.03^{a}	0.104 ± 0.095	6.48 ± 6.0
Native Riparian	2.78 ± 0.82^{a}	0.302 ± 0.09^{a}	0.048 ± 0.018	2.12 ± 0.96
Poplar Plantation	2.37 ± 0.09^{a}	0.772 ± 0.07^{b}	0.073 ± 0.013	3.10 ± 0.68
Baseflow				
Gravel Bar	1.61 ± 0.18^{a}	0.191 ± 0.05^{a}	0.026 ± 0.018	1.43 ± 0.95
Native Riparian	1.31 ± 0.62^{a}	1.17 ± 0.47^{c}	0.070 ± 0.040	4.85 ± 0.93
Poplar Plantation	4.03 ± 0.23^{b}	0.258 ± 0.03^{a}	0.368 ± 0.20	8.87 ± 0.49

FIGURE LEGENDS

Figure 1. The Garonne River catchment and location of study segment.

Figure 2. Detail of Monbéqui study site with piezometer location and vegetation type.

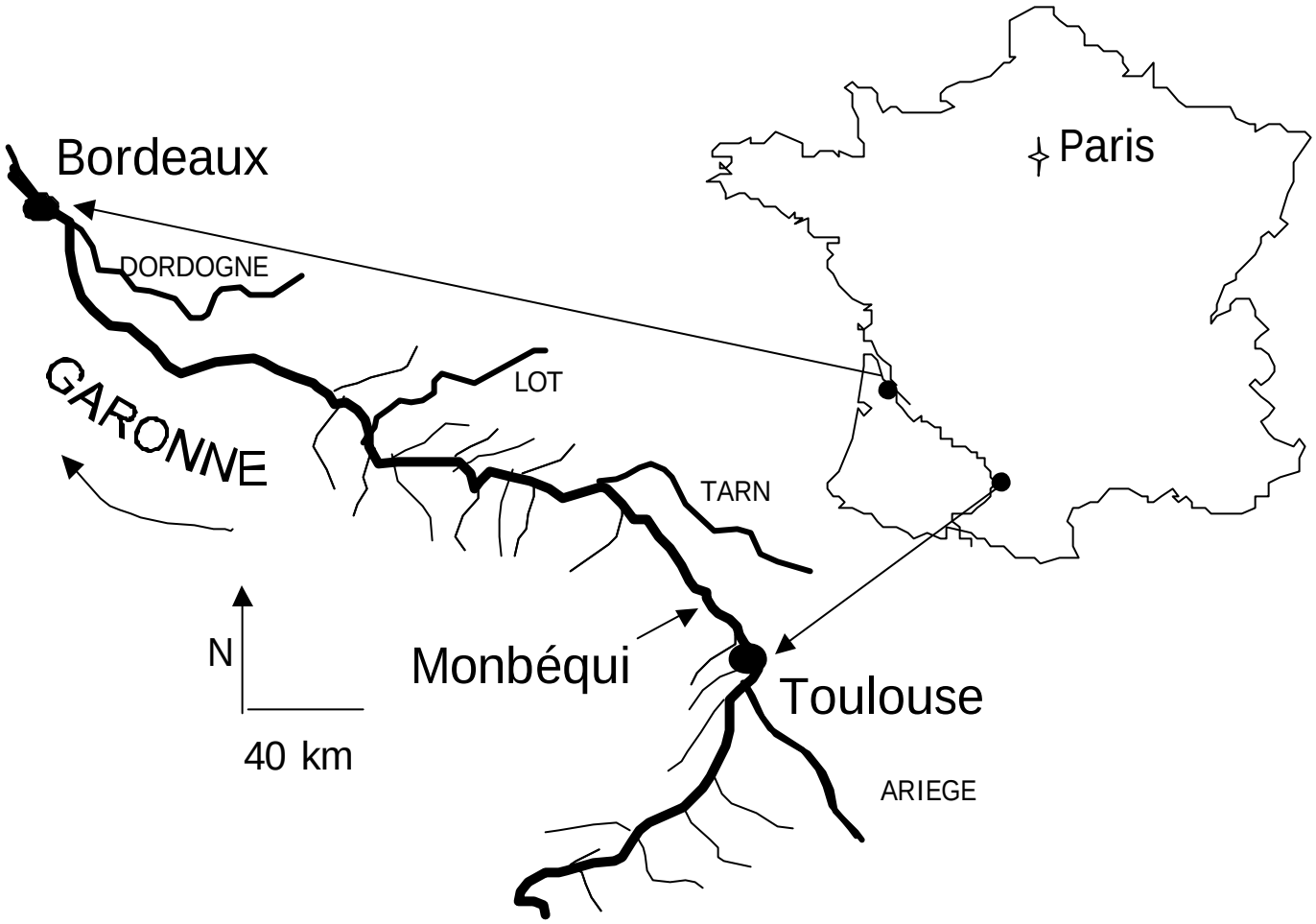
Figure 3. Garonne River hydrograph during water year October 1998- 1999. Timing of intensive sampling indicated in circles.

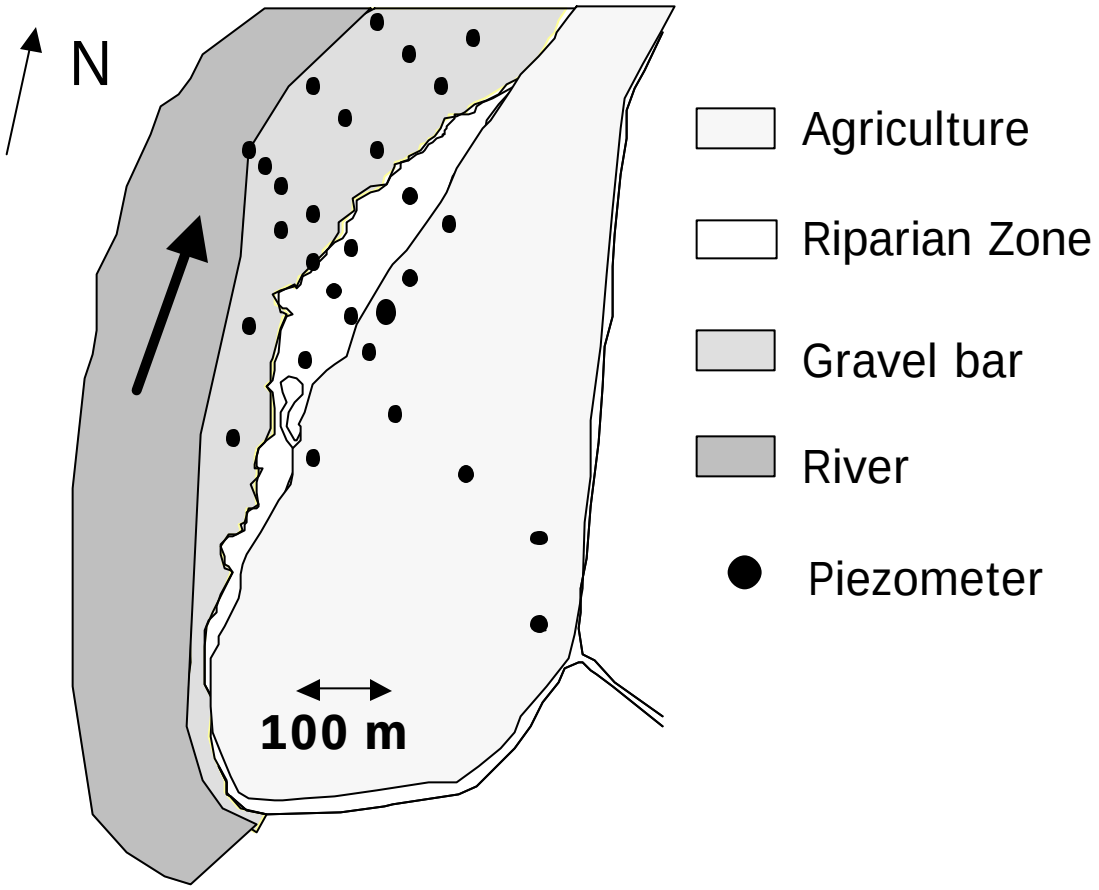
Figure 4. Schematic of the *in-situ* push-pull acetylene block technique during push phase. An inflatable bladder sealed a 20 cm section of well screen for measurements at discrete depths below the water table. Acetylene was generated in the field using calcium carbide and bubbled into the injection solution until the water was saturated with the gas.

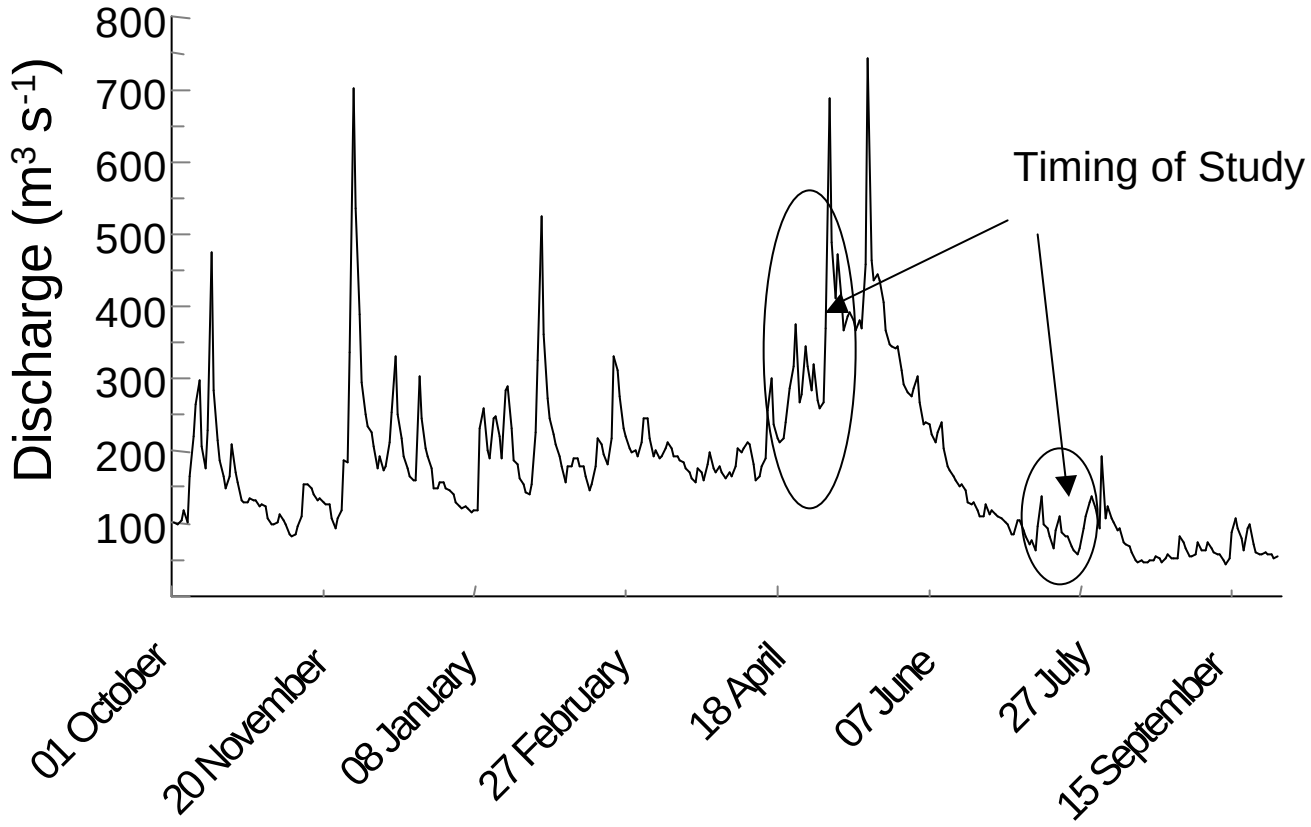
Figure 5. Typical *in-situ* push-pull acetylene block results showing production of N_2O (filled circles and solid line) and NO_3-N consumption (open circles and dashed line).

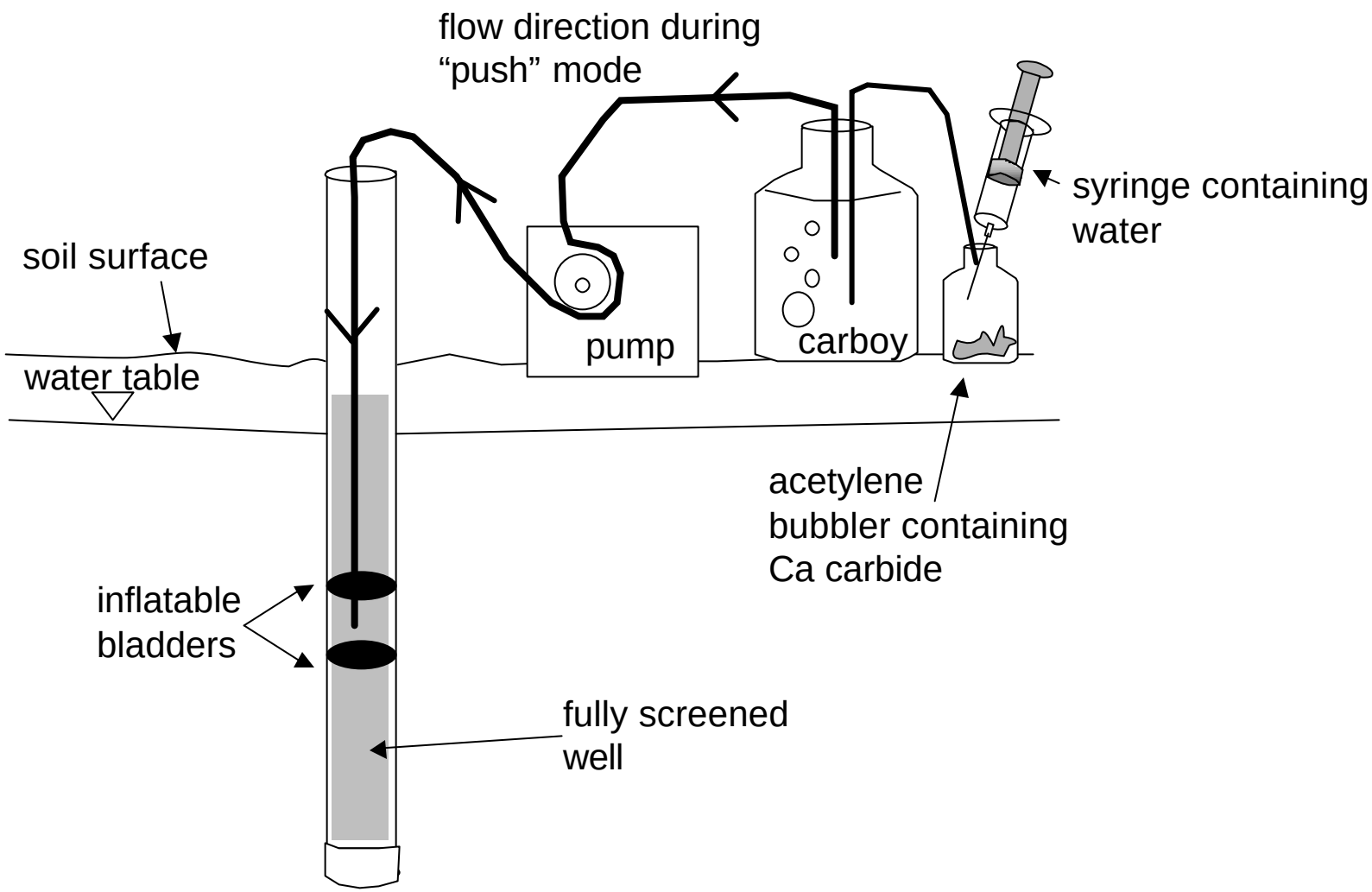
Figure 6. Relationship between observed nitrate concentration and nitrate concentration predicted by end-member mixing analysis (EMMA). Points above the 1:1 mixing line indicate nitrate retention while points below the line indicate nitrate production.

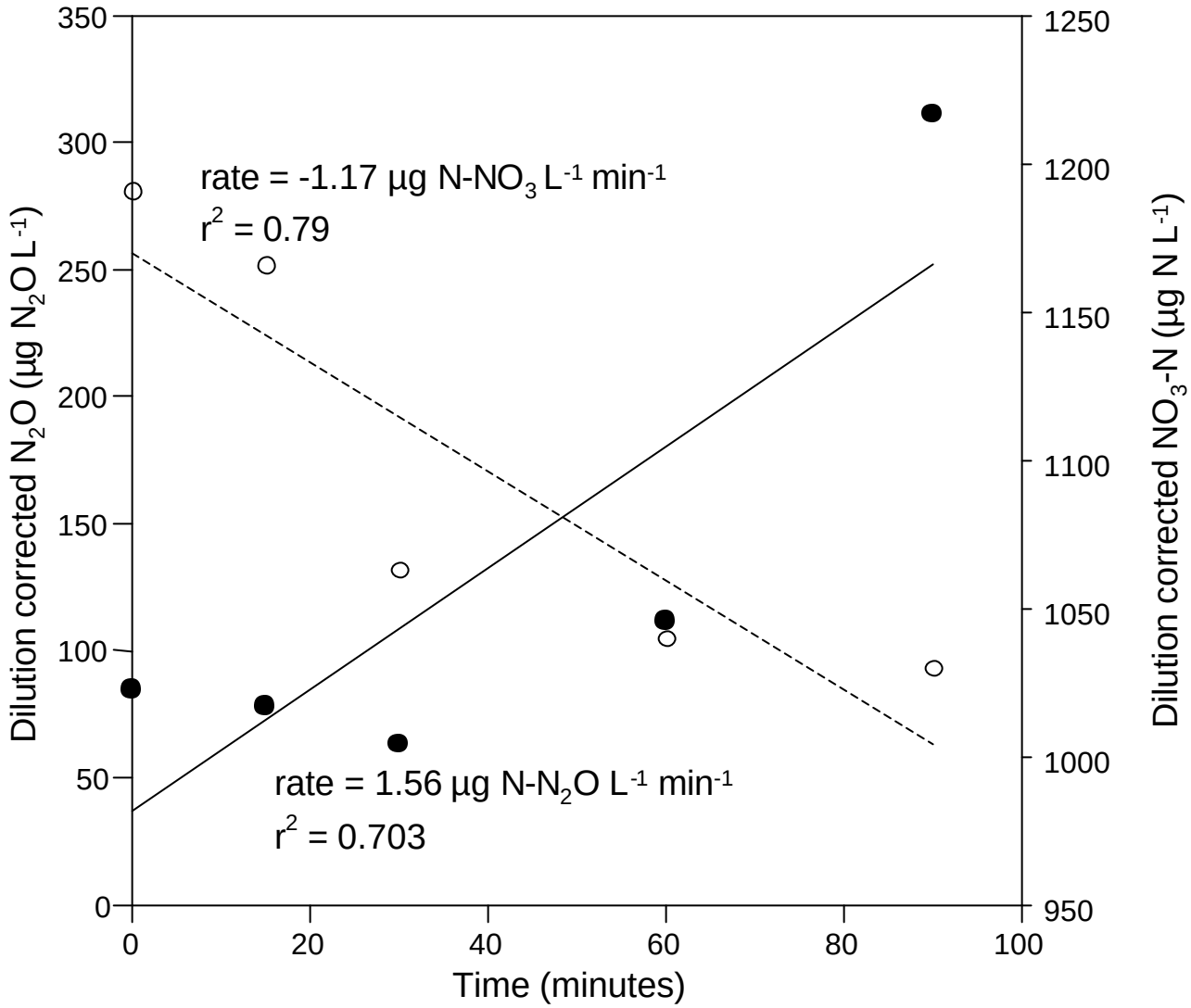
Figure 7. Mean (+SE) denitrification rates in three vegetation types during spring high flow and summer base flow. Significantly different means (repeated ANOVA, $p<0.05$) are indicated by an asterisk.


Bordeaux
Paris
DORDOGNE
LOT
GARONNE
TARN
N
Monbéqui
Toulouse
40 km
ARIEGE

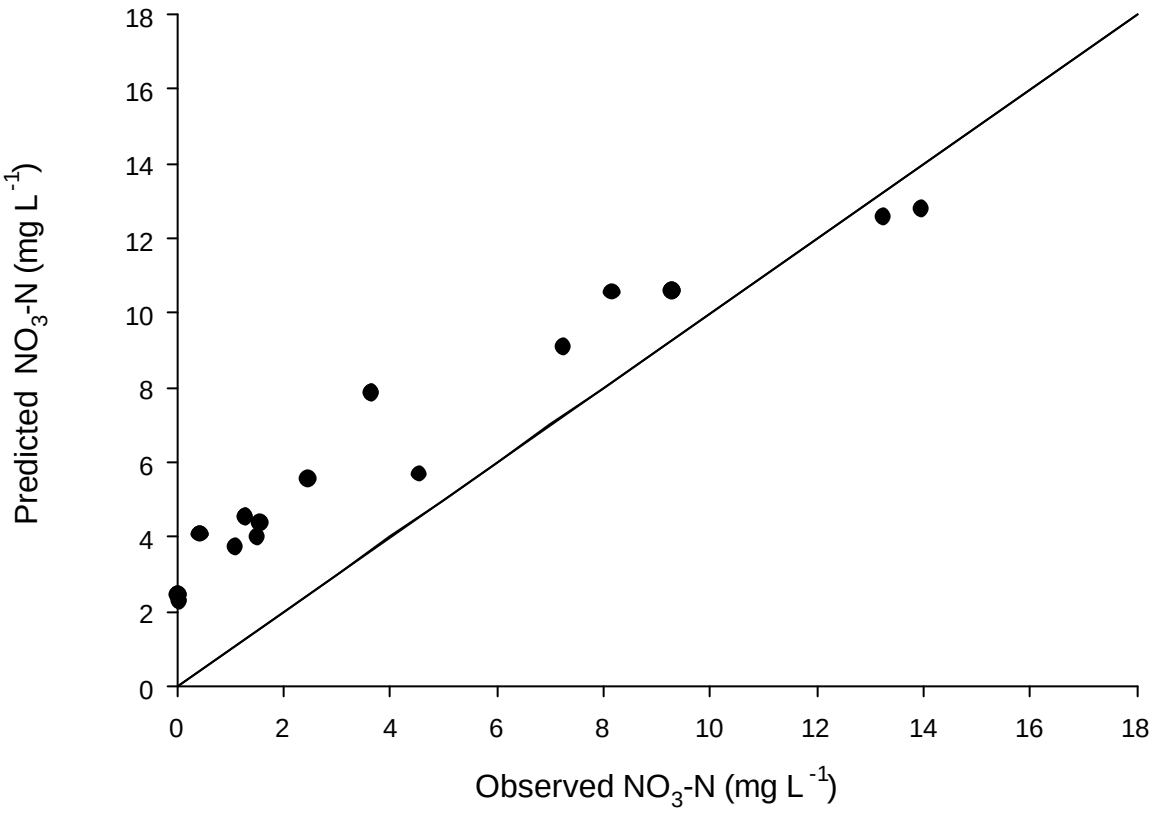
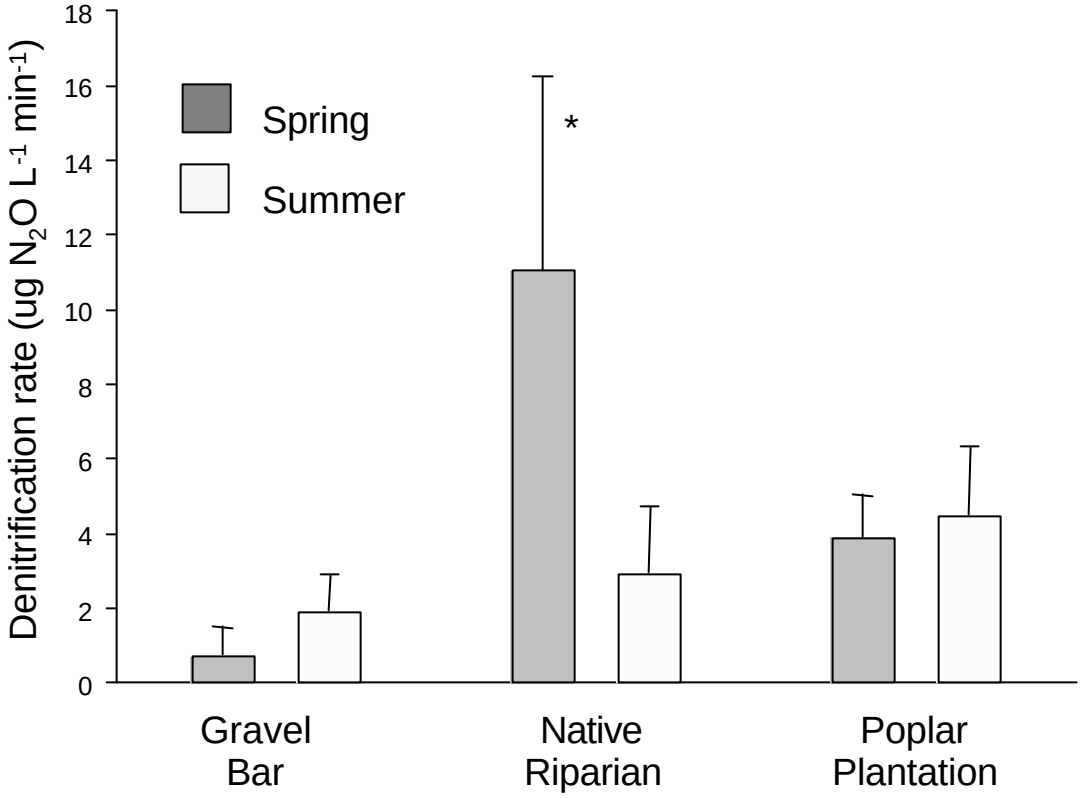

N
100 m
Agriculture
Riparian Zone
Gravel bar
River
Piezometer


Discharge (m3 s-1)
800
700
600
500
400
300
200
100
Timing of Study
01 October
20 November
08 January
27 February
18 April
07 June
27 July
15 September


flow direction during
“push” mode
syringe containing
water
soil surface
pump
carboy
water table
acetylene
bubbler containing
Ca carbide
inflatable
bladders
fully screened
well


rate = -1.17 µg N-NO3 L-1 min-1
r2 = 0.79
rate = 1.56 µg N-N2O L-1 min-1
r2 = 0.703
Dilution corrected N2O (µg N2O L-1)
Dilution corrected NO3-N (µg N L-1)
Time (minutes)
0
50
100
150
200
250
300
350
950
1000
1050
1100
1150
1200
1250
0
20
40
60
80
100

Baker & Vervier Figure 6



Baker & Vervier Figure 7