Spatial variability of denitrification along a nitrate-rich seepage chain of lakes (Ruidera Natural Park, Central Spain)

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ABSTRACT

Spatial variability of denitrification activity and the relative importance of controlling factors were determined using the isotope pairing technique in a seepage chain of Mediterranean flowthrough lakes (Ruidera lakes) and within a given lake in the chain (Colgada lake), all receiving extremely high nitrogen inputs. The range of denitrification rates measured (28-155 µmol N m⁻² h⁻¹) were comparable with rates measured with isotope pairing in other freshwater systems. While the bulk of total denitrification was mostly based on NO₃⁻ from the overlying water, coupled nitrification-denitrification seemed to be lower in all Ruidera lakes. At the regional scale, i.e. lake district, inter-variability in denitrification (CV = 37 %) was lower than the intra-variability observed in a single lake (CV = 54 %). In fact, a preliminary meta-analysis of data from published studies suggested that denitrification rate variabilities did not differ statistically between the environmental and regional scales. Dissolved oxygen concentration, dissolved organic carbon content and nitrate from the overlying water were found to be the most important factors affecting the spatial heterogeneity of denitrification rate in the lake complex. At the single-lake scale, only phosphorus explained the variability of denitrification rates observed along its main axis. Our study emphasizes the significance of hotspots in denitrification processes, but also highlights the need for further studies on spatial denitrification given that fact that conflicting controlling factors have been found at different scales. To spatially measure denitrification is complicated due to the intrinsic difficulties of the incubation process and hotspots, which makes modelling indispensable.

Key words: isotope pairing technique, lake district, controlling factors, hotspots, meta-analysis

RESUMEN

Variabilidad espacial de la desnitrificación en una cadena de lagos rica en nitrato y con alimentación subterránea (Ruidera, Centro de España)

Mediante la técnica del par isotópico, estudiamos las variaciones espaciales de la actividad desnitrificante y la importancia relativa de sus factores de control en una cadena de lagos mediterráneos de tipo fluvial (lagunas de Ruidera) y en un lago concreto dentro de la misma (Laguna Colgada), todos los cuales reciben cargas elevadas de nitrógeno. El rango de las tasas medidas mediante dicha técnica fue comparable (28-155 µmol N m⁻² h⁻¹) al registrado en otros ambientes dulceacuícolas. Gran parte de las tasas se debieron al nitrato presente en el agua sobrenadante al sedimento (62-83 %), resultando de menor importancia los procesos acoplados de nitrificación-desnitrificación en todos los lagos estudiados. A escala del conjunto lacustre, la variabilidad del proceso fue inferior (CV = 37 %) a la presente en un único lago (CV = 54 %). En relación con esto, un meta-análisis preliminar de la variabilidad de la desnitrificación en distintos linnoambientes reveló que no había diferencias significativas entre la escala del ecosistema individual y la regional. Las concentraciones de oxígeno disuelto, carbono orgánico disuelto y nitrato en el agua sobrenadante fueron los factores fundamentales que controlaron las tasas de desnitrificación en el conjunto de lagos, mientras que en la laguna Colgada solo el ortofosfato explicó la variabilidad del proceso registrada en su eje principal. Este estudio recalca la importancia de los micrositios más favorables (hotspots) para el proceso de desnitrificación, pero también apoya la necesidad de más estudios sobre el tema porque los factores que lo
INTRODUCTION

There is little doubt that reactive nitrogen fixed by human activities has risen substantially in the past few decades. Consequently anthropogenic N is accumulating in aquatic ecosystems and altering many ecological processes (Galloway et al., 2003; Rabalais, 2002; Vitousek et al., 2002).

Denitrification (microbial production of N\textsubscript{2} from nitrate and nitrite) is the only process that permanently removes reactive N from the environment. There are many denitrification studies across different aquatic ecosystems (Piña-Ochoa & Álvarez-Cobelas, 2006); however, few locations have sufficient measurements to quantify rates, or to understand factors controlling denitrification at the ecosystem scale (Seitzinger et al., 2006). Nowadays, data are available on denitrification activity and its controlling variables across different ecosystems (Cornwell et al., 1999; Mengis et al., 1997; Risgaard-Petersen, 2003; Mulholland et al., 2004; Risgaard-Petersen et al., 2013) but studies on terrestrial environments far outnumber those on freshwaters (Groffman et al., 2009). Furthermore, denitrification studies regarding spatial heterogeneity are limited and studies using the landscape approach are still scarce. The main reason for this is that field measurements of denitrification are notoriously complicated due to the technical difficulties of measuring N\textsubscript{2} production and, even more difficult to resolve, the large spatial variation of process rates involved (Seitzinger et al., 2006). The large spatial variability in production of N\textsubscript{2} is influenced by a series of environmental factors, each of which is subject to spatial variability. Therefore, we need to gain a better understanding of variability sources and factors controlling denitrification at the ecosystem level. To date, most studies on the spatial variability of denitrification activity are site-specific and have been performed in streams, alluvial valleys and coastal ecosystems (García-Ruiz et al., 1998; Tuominen et al., 1998; Nielsen et al., 2001; Dong et al., 2000; Orr et al., 2014; Wang et al., 2017). In lakes, however, these studies are still scarce, albeit growing in recent years (Bruesewitz et al., 2012; Rissanan et al., 2011, 2013; Wang et al., 2017).

A combination of factors, which jointly vary, may explain the greatest amount of spatial variation in denitrification. N loading was shown to influence denitrification rates (García-Ruiz et al., 1998; Nielsen et al., 2001; Wall et al., 2005). Anoxic conditions can constitute a factor controlling the spatial variation in deep areas of the ocean (Tuominen et al., 1998). In an extensive spatial coverage of a coastal ecosystem, Nowicki et al. (1998) attributed spatial differences in denitrification rates to differences in sediment type and organic content. The highest denitrification activity was very often attributable to higher biomasses of benthic fauna (Tuominen et al., 1998; Risgaard-Petersen, 2003; Rysgaard et al., 1995; Nowicki et al., 1998), but it is also dependent upon submerged plant compositions (Caffrey & Kemp, 1990; Vila-Costa et al., 2016). At the ecosystem scale, geology and hydrology interact to control the residence time of water and thus the processing time of N within an aquatic ecosystem (Seitzinger et al., 2006) and hence the proportion of N that can be denitrified. In the Iberian Peninsula, there are still few studies on aquatic denitrification (Piña-Ochoa, 2007; Castellano-Ortigosa et al., 2017; Vila-Costa et al., 2016) while none at all address heterogeneity at different spatial scales.

Here, we explore the spatial intra- and inter-variability in denitrification in a lake district affected by massive N inputs at two spatial scales: among lakes and within a given lake. We also relate the process variability to the variability of likely controlling factors. Also, a compilation of
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**Figure 1.** Site location. The Ruidera lake chain where studies in several lakes were undertaken; it runs from the upper ones in the south-eastern area to the lower ones in the north-western area of the catchment. Lugar de estudio. Cadena de lagunas de Ruidera, desde las más altas en la zona SE a las más bajas en la NW.
studies delving into spatial variability of denitrification rates enable us to perform a preliminary meta-analysis of such variability at the local and the regional scale.

**MATERIALS AND METHODS**

**Study area**

The Ruidera Lakes Natural Park is located in the Campo de Montiel area of Central Spain (40° 55’ N, 5° 40’ W, Fig. 1). The extension of the geographical area is 4937 km², whereas that of groundwater catchment is 2575 km². Surface catchment is smaller, roughly attaining 800 km². Water is drained to three major watersheds, but that of the Guadiana is by far the largest one. The Ruidera Lakes are comprised by 18 natural basins and a man-made reservoir (Fig. 1). These water bodies are chain-connected, small (0.1-103 ha), shallow or moderately deep (0.5-10.4 m of average depth; Alvarez-Cobelas et al., 2006a). They run SE-NW and are flowthrough, groundwater- and riverine-fed lakes, while Peñarroya reservoir, used for irrigation purposes, stores downstream water drained from both the lakes and the groundwater aquifer.

The order of lakes studied from upstream to downstream is the following: Conceja, San Pedro, Lengua, Colgada, Rey and Cueva Morenilla (Fig. 1). All lakes are warm monomictic and usually stratify from May to October. Most lakes are very similar hypsographically, being convex, except the shallower ones that are more concave. Groundwater inputs feed all lakes, and these may take place through point-source (e.g. subaquatic springs) and/or diffuse flows (Table 1); hence this could be another source of within- and among-lake variability. All lakes have alkalinites ranging between 2.15-4.38 meq/L and are bicarbonate and calcium-rich environments. Water renewal time of these lakes fluctuates greatly inter-annually (Álvarez-Cobelas et al., 2006b), but during this study period it was much higher than a year (Table 1) when low rainfall made both groundwater level and stream discharge decrease, and hence water volume diminished for most of the lakes under study.

Ruidera lakes are heavily polluted by nitrate inputs through groundwater, whose main origin is agricultural irrigation, with total nitrogen concentrations of 7-17 mg N/L, of which 75-90% is nitrate. However, P is the main limiting factor and this results in oligo- to mesotrophic status of lakes due to the small P concentrations involved (see dissolved N:P ratios in Table 1).

Spatial variability in denitrification in Colgada Lake was surveyed at five equidistant sites along the SE-NW main axis. We would expect some heterogeneity in denitrification activity because this lake receives surface water from the upper lake, two subaquatic and seven surface springs, and through diffuse groundwater input where regional groundwater flows preferably in the SE-NW direction. All Ruidera lakes usually harbour submerged populations of macrophytes (Álvarez-Cobelas et al., 2006a), which might influence processes like denitrification (Caffrey & Kemp, 1990), but they were largely absent from the main axis during the study because they were too deep to support macrophyte photosynthesis.

**Sampling and laboratory incubations**

Six lakes were studied (Conceja, San Pedro, Lengua, Colgada, Rey and Cueva Morenilla) in July 2005. Sampling was undertaken in the central area of each lake, except for Colgada Lake which was surveyed in five different, equidistant sites along its main axis. Seemingly, these sites were environmentally similar, as judged by dissolved oxygen profiles (Álvarez-Cobelas, unpublished data) and visual inspection of cores. Vertical profiles of dissolved oxygen and water temperature were recorded in situ with a Yellow Springs ODO probe at all sites from where sets of cores were retrieved.

The rate of denitrification was determined using the isotope pairing technique (IPT, hereafter; Nielsen, 1992). Sets of eight sediment cores (Plexiglas cylinders, 5.5 inner diameter, 60 cm height) with 10 to 22 cm column of sediments were collected at the deepest part of each sampling site (i.e. eight cores per lake in the among-lakes’ study and eight cores per site in the within-lake study) and transferred to the laboratory in thermoboxes within 5 h. Care was taken to preserve sediment structure during sampling and

transport. Bottom water for incubation was pumped from 0.5 m above the bottom and transported in plastic containers.

In the laboratory each set of sediment cores was opened and immersed in an incubation tank filled with 10 L bottom water of each site (from either each lake or within-site for the Colgada Lake study), thermostated at in situ temperature. The water column in each core was mixed by small Teflon coated magnets driven by an external magnet (30 r.p.m.), placed 6 cm above the sediment surface. The O$_2$ concentration was maintained near in situ levels by gentle purging with a N$_2$/air gas mixture. Prior to incubation experiments, cores were preincubated in the dark at in situ temperature for 2 h. When cores had been thermally stabilized in the tank, incubation was initiated by adding 3.3 ml of a labelled 0.05M K$_{15}$NO$_3$ stock solution (98 atom %, $^{15}$NO$_3$, Sigma Ltd, UK) into the cores to final concentrations ranging from 480 to 935 µM NO$_3$ and the cores were left to equilibrate the overlying water with pore water. Such a wide range resulted from the wide range of natural NO$_3$ concentration occurring in waters of each set of cores collected at different sites. Such a wide range of resulting $^{15}$N addition did not affect results because this addition was done in excess regarding the expected isotopic signals after incubations. The enrichment of $^{15}$N in the water column after isotope addition was 3 %.

After 40 min, cores were closed with rubber stoppers having ensured complete mixing of bulk water of the corresponding site at which each core had been retrieved. NO$_3$ concentration in the overlying water was measured before and after $^{15}$NO$_3$ addition to estimate $^{15}$N enrichment. At the initial point of incubation, two samples for time zero were collected as reference cores, and the remaining cores were incubated for 48h. After incubation end stoppers were removed, 250 µL of a 50 % (w/w) ZnCl$_2$ solution were added to each core to stop bacterial activity, resulting in a final concentration of 0.012 % (w/w) of ZnCl$_2$ in each core. Sediment and water phases were shaken vigorously for several minutes. A sample of resultant sediment slurry was taken with a syringe.

Water samples for nitrate (NO$_3$ hereafter), Dissolved Organic Carbon (DOC hereafter), Soluble Reactive Phosphorus (SRP hereafter) and $^{15}$N$_2$ analyses were rapidly taken. Nitrate concentration was too high, therefore the contribution of ammonia as substrate for the nitrification coupling to denitrification process was considered negligible. Dissolved oxygen in each core was measured with a Yellow Springs ODO probe. Slurry samples for $^{29}$N$_2$ and $^{30}$N$_2$ were stored in gas tight containers (Exetainers, Labco, High Wycombe, UK) containing a few drops of ZnCl$_2$ solution (50 %, w/w). Entrapment of air in the extainer was carefully avoided.
Analysis and calculations

All nutrient analyses (NO3, DOC, SRP) of water samples were determined following Standard Methods (APHA, 1998). Water retention time of each lake in July 2005 was estimated knowing maximal lake volumes (Table 1, and see Álvarez-Cobelas et al., 2006), water inputs and changes in lake levels (which enabled to estimate lake volume variation) in the previous year, these latter variables being recorded by the Guadiana Water Authority and the staff of Lagunas de Ruidera Lake District.

The concentrations of excess 29N2 and 30N2 in the slurry samples were determined with IPT using a gas chromatograph connected with a Fisons Optima mass spectrometer in the EEZ (CSIC)-stable isotope laboratory. The analytical reproducibility was ± 0.1 % for δ15N, determined from repeated analyses of international nitrogen isotope standards (IAEA-N1 and IAEA-N2).

The 15N2 (15N15N and 14N15N) of samples was extracted into a helium headspace in the exchangers. After 5 min of vigorously shaking most N2 was found in the headspace. The gas in the headspace was then injected into a gas chromatograph coupled to the mass spectrometer and the abundance and concentrations of 14N15N and 15N15N were analysed. The gas was purified in a glass column packed with Carbosorb. Additionally, the gas flow was led through a heated copper reduction tube (600 °C).

For further calculations, the interstitial water volume (viw) of the sediment column of each core was determined as:

\[ viw = ((ww-dw)/d) \]

where \(ww\) and \(dw\) were the wet- and dry weight of each sedimentary sample, respectively, and \(d\) was sediment density. Dry weight was determined after drying sediments at 105 °C in an oven until constant weight. Sediment density was determined as wet weight of sediment sample/volume of wet sediment sample.

Denitrification rates were estimated from the production of 15N isotopes (Nielsen, 1992), using the following formulae:

\[ D_{15} = (14N15N) + 2 \cdot (15N15N) \]  
\[ D_{14} = [(14N15N)/(2 \cdot (15N15N))] \cdot D_{15} \]  
\[ D_{\text{tot}} = D_{14} + D_{15} \]

where \(D_{15}\) and \(D_{14}\) were the rates of denitrification based on 15NO3 and 14NO3, respectively, and \((14N15N)\) and \((15N15N)\) were the rates of production of the two labelled N2 species \((14N15N)\) and \((15N15N)\), respectively. While \(D_{15}\) expressed denitrification activity of added 15NO3, \(D_{14}\) expressed in situ total denitrification activity. The denitrification rate obtained was reported in μmol N m⁻² h⁻¹ by multiplying \(D_{14}\) by the total water volume sample (± volume of water phase + volume of interstitial water phase, L) and by dividing it by the surface area (m²) of the sample and the incubation time (h). The proportion of \(D_{14}\) based on NO3 from the water phase \((D_w)\) was calculated from \(D_{15}\) and the 14N:15N ratio of the water column NO3 as follows:

\[ D_w = D_{15} \frac{[14NO3]_w/[15NO3]_w} \]

where \([14NO3]_w\) was the concentration of 14NO3 and \([15NO3]_w\) was the concentration of 15NO3 in the water column. Finally, in situ denitrification of NO3⁻ arisen from nitrification \((D_n)\) was calculated as:

\[ D_n = D_{14} - D_w \]

Since denitrification rates among cores are somewhat variable, we decided to undertake simple, non-parametric relationships between them and the likely controlling factors of the denitrification process, using the Spearman correlation approach (Siegel & Castellan, 1988). Except for water renewal time, which was estimated at the single-lake scale, all remaining environmental factors were those averaged after being measured during core incubations. In order to examine those correlations after partialling out for one or more variables, partial correlations were undertaken with environmental controlling factors of denitrification. All statistical calculations were performed with the STATISTICA7 package.
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RESULTS

Figure 2 shows the spatial variation of denitrification rates at five different sites in Colgada Lake during July 2005. While rates of total denitrification (D_{tot}) ranged from 31 ± 8 µmol N m^{-2} h^{-1} to 155 ± 17 µmol N m^{-2} h^{-1}, both stations being close at the SE end of the lake, denitrification rates in the water column ranged from 25 ± 20 µmol N m^{-2} h^{-1} to 112 ± 8 µmol N m^{-2} h^{-1}. There was a statistically significant spatial difference for both types of denitrification (U-test, p < 0.05) across all sampling stations, which were otherwise uncorrelated with each other (p > 0.05). Water-phased nitrate denitrification did not correlate with NO_{3} concentration in the overlying water (p > 0.05, t-test).

Total denitrification rates (D_{tot}) in the Ruidera lake chain ranged from 28 ± 41 in Rey Lake to 89 ± 21 µmol N m^{-2} h^{-1} in Conceja Lake (Fig. 3). However, despite the general decrease in NO_{3} concentration along the lake chain from high to low altitude in the catchment, there was no concomitant pattern in denitrification rates. Nitrate from the overlying water was again the main source of NO_{3} for denitrification, with D_{w} representing 62–83% of total denitrification rates, and its average rate was 74 ± 8 µmol N m^{-2} h^{-1}. Coupled nitrification-denitrification within the sediment (D_{n}) was negligible in all lakes, thus suggesting that denitrification based on water-phase NO_{3} played the main role in nitrogen removal in all lakes. Denitrification based on water NO_{3} showed good correlation (R^2 = 0.47, p < 0.05) with NO_{3} concentration in the overlying water.

In the study of spatial heterogeneity of Colgada Lake, denitrification rates were significantly and positively correlated with SRP (Fig. 4; R^2 = 0.68, p < 0.05). The highest SRP concentration occurred at the south-eastern site, which also reached the highest denitrification rates in the lake. DOC, NO_{3}, dissolved oxygen and water residence time had no significant correlation with denitrification (p > 0.05). No partial correlations among controlling variables were found to be statistically significant (p > 0.05)

Several factors have been shown to influence denitrification activity in the Ruidera Lake chain (Fig. 5). DOC content was significantly and positively correlated (R^2 = 0.56, p < 0.05) with denitrification rates. The highest organic carbon content occurred in Conceja and Colgada lakes, which had the highest denitrification rates, whereas the lowest denitrification rates corresponded to low organic carbon content of Rey Lake. The denitrification rate showed a negative
correlation with dissolved oxygen in all lakes ($R^2 = 0.50$, $p < 0.05$). Also, a positive significant correlation was found between overlying water NO$_3$ and denitrification ($R^2 = 0.59$, $p < 0.05$). The denitrification rate did not show a significant correlation ($p > 0.05$) with any other variable in the correlation analyses; more specifically, water renewal was unrelated with denitrification rates. No partial correlations among controlling variables of denitrification across the lake chain were statistically significant ($p > 0.05$).

The inter-variability in denitrification along the lake chain ($CV = 37\%$) was significantly lower ($U$-test, $p < 0.05$) than the intra-variability in the single lake ($CV = 54\%$).

**DISCUSSION**

**Denitrification rates**

It has previously been stated that coupled nitrification-denitrification may be the major source of nitrate for denitrification (Seitzinger, 1988; Lohse et al., 1996; Sundbäck and Miles, 2002; Wang et al., 2003; Tuominen et al., 1998), while the water column accounts for 60% or more of the nitrate for denitrification in other instances (Rysgaard et al., 1995; Nielsen et al., 2001; Dong et al., 2000). Our study indicates the opposite, i.e., uncoupled denitrification ($D_w$) accounts for a very high fraction of the whole process in Ruidera lakes, a system receiving extremely high nitrogen inputs, whereas coupled nitrification-denitrification did not seem to contribute much to total denitrification. There is considerable variation, however, in the relative importance of both processes, where NO$_3$ concentration in the water overlying sediments can largely explain the wide variability in either the proportion of sediment denitrification supported by coupled nitrification-denitrification or the nitrate content in overlying water. In systems with low nitrate concentrations in bottom water (< 10 µM), coupled nitrification-denitrification accounts for 90% or more of the nitrate required to support denitrification. At nitrate concentrations higher than 60 µM, bottom water becomes the dominant source of nitrate, accounting for 80% of the overall nitrate required (Seitzinger et al., 2006).

As a whole, the range of denitrification rates measured within and among Ruidera lakes (28-155 µmol N m$^{-2}$ h$^{-1}$) were comparable with the rates measured with IPT in other freshwater, estuarine and marine environments (Piña-Ochoa & Álvarez-Cobelas, 2006), although they were in the upper range. Macrophyte occurrence and composition, previously suggested as mediating factors in this process (Caffrey & Kemp, 1990; Vila-Costa et al., 2016), can be ruled out as a cause of denitrification variability in Ruidera lakes because they were absent at the main axes of lakes when this study was undertaken.

**Controlling factors**

We still lack a comprehensive, quantitative understanding of denitrification rates and controlling factors across ecosystems (Davidson & Seitzinger, 2006). Furthermore, we suspect that spatial differences in denitrification between lake districts mainly connected by surface waters and those connected by groundwater might be controlled by different sets of variables, also acting differently at the local and the regional scale. Hence, at the lake chain scale, spatial variability in sediment denitrification appeared to be driven by DOC content, O$_2$ concentration, and NO$_3$ content in the overlying water of these seep-
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Figure 5. Controlling factors of denitrification in the Ruidera lake chain in July 2005, as judged by both a linear regression and the Spearman correlation analyses. All data used in statistical analyses are averages. Factores de control de la desnitrificación en el conjunto de las lagunas estudiadas de Ruidera en Julio de 2005, analizados mediante regresión lineal y el método de correlación de Spearman. Todos los datos usados en el análisis estadístico son promedios.

Our results showed that DOC was positively correlated with denitrification rates, where the highest activity occurred in lakes with higher organic carbon content (Concejada and Colgada). This control by DOC was likely the result of DOC compounds’ use as electron donors, while NO₃ served as oxidant. Similar results were reported by Nowicki et al. (1997) and Mitchell and Baldwin (1999), the latter demonstrating a very strong increase in denitrification rates when organic carbon was added to lake sediments. On the contrary, Nielsen et al. (2001) could not find such a significant correlation. Denitrification showed a significant negative correlation with dissolved oxygen in all sampled lakes, as also reported by Tuominen et al. (1998). Oxygen-regulated denitrification in lake sediments was thoroughly investigated by Rysgaard et al. (1994).

At Concejada and Colgada lakes, where NO₃ content was the highest among all lakes, denitrification rates were also the highest. Such a straightforward relationship between NO₃ and denitrification is consistent with previous reports (Nielsen et al., 2001; Rysgaard et al., 1995; Dong et al., 2000; Wall et al., 2005; Straus et al., 2006). Water residence time, partly associated with groundwater flux and landscape position, seemingly help to explain divergences in ecosystem response (Webster et al., 2000). However, water residence time did not explain denitrification variability in the lake complex, perhaps because of the lengthy water renewal time observed during our experiments. However, we did not find the same pattern of spatial denitrification observed for nitrate along the lake chain in the single lake.

Heterogeneity at different spatial scales

There are very few studies that thoroughly investigate the spatial heterogeneity of denitrification in whole systems or at the ecosystem level. Our study shows that intra-variability in denitrification activity along the main axis of Colgada Lake was high (CV = 54 %), this variability being higher than inter-variability found along the Ruidera lake chain (CV = 37 %). Higher CV of rates as the spatial scale increases was expected since inter-lake variability is normally higher and may be driven by different factors acting at both regional (regional landscape characteristics, climate) and local scale (lake morphometry, hydrology, foodwebs) whereas intra-lake variability is normally attributed to more site-specific factors which can decrease denitrification variability. Some studies (Nowicki et al., 1997; Dong et al., 2000; Tuominen et al., 1998) found significant spatial denitrification differences. However, other studies (Seitzinger et al., 1984; Nielsen et al.,...
not seem to contribute much to total denitrification of the whole process in Ruidera lakes, a correlation analyses; more specifically, water draining from both the lakes and the groundwater, whose main origin is the mass balance approach. Variabilidad espacial (estimada como coeficiente de variación) de la desnitrificación no enriquecida con nutrientes, en ambientes acuáticos de todo el mundo. C2H2: técnica del acetileno, CV: coeficiente de variación, IPT: técnica del par isotópico, MIMS: espectrometría de masas con entrada de membrana, SD: desviación típica.


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<th>SD (%)</th>
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<tr>
<td>River</td>
<td>Changjiang (upriver)</td>
<td>China</td>
<td>2</td>
<td>MIMS</td>
<td>198</td>
<td>19</td>
<td>10</td>
<td>mg N m⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland</td>
<td>Nanjishan</td>
<td>China</td>
<td>9</td>
<td>MIMS</td>
<td>206</td>
<td>59</td>
<td>29</td>
<td>mg N m⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland</td>
<td>Wucheng</td>
<td>China</td>
<td>10</td>
<td>MIMS</td>
<td>166</td>
<td>34</td>
<td>20</td>
<td>mg N m⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>Baraboo River (large plots)</td>
<td>USA</td>
<td>81</td>
<td>C₂H₂</td>
<td>59</td>
<td>33</td>
<td>5</td>
<td>ng N g⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>Baraboo River (small plots)</td>
<td>USA</td>
<td>36</td>
<td>C H</td>
<td>1.69</td>
<td>1.42</td>
<td>84</td>
<td>ng N g⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>Teaneck Creek</td>
<td>USA</td>
<td>10</td>
<td>C₂H₂</td>
<td>1.69</td>
<td>1.42</td>
<td>84</td>
<td>ng N g⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>Wisconsin River (large plots)</td>
<td>USA</td>
<td>81</td>
<td>C₂H₂</td>
<td>105</td>
<td>51</td>
<td>51</td>
<td>ng N g⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>Wisconsin River (small plots)</td>
<td>USA</td>
<td>36</td>
<td>C₂H₂</td>
<td>51</td>
<td>51</td>
<td>51</td>
<td>ng N g⁻¹ h⁻¹</td>
</tr>
</tbody>
</table>
Spatial variability of denitrification

Table 3. Spatial variability (as CV) of unamended denitrification rates in districts of freshwater environments worldwide. Abbreviations as in Table 2. Variabilidad espacial (estimada como coeficiente de variación) de la desnitrificación no enriquecida con nutrientes, a la escala regional de ambientes acuáticos en todo el mundo. Las siglas, como en la Tabla 2.

<table>
<thead>
<tr>
<th>District</th>
<th>Country</th>
<th>Number of ecosystems</th>
<th>DN assessment technique</th>
<th>Average denitrification (mg N m⁻² h⁻¹)</th>
<th>CV (%)</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fayetteville</td>
<td>USA</td>
<td>3</td>
<td>N₂ measurement</td>
<td>0.030</td>
<td>15</td>
<td>µg N g⁻¹ h⁻¹</td>
<td>Small et al. (2016)</td>
</tr>
<tr>
<td>Great Lakes</td>
<td>USA-Canada</td>
<td>3</td>
<td>C₂H₂</td>
<td>0.004</td>
<td>15</td>
<td>µg N g⁻¹ h⁻¹</td>
<td>Rissinan et al. (2013)</td>
</tr>
<tr>
<td>Rotorua</td>
<td>New Zealand</td>
<td>10</td>
<td>C₂H₂</td>
<td>18.9</td>
<td>119</td>
<td>µmol N m⁻² d⁻¹</td>
<td>Brusewitz et al. (2011)</td>
</tr>
<tr>
<td>Raudera</td>
<td>Spain</td>
<td>6</td>
<td>IPT</td>
<td>22</td>
<td>37</td>
<td>µmol N m⁻² d⁻¹</td>
<td>This study</td>
</tr>
<tr>
<td>Southern Finland</td>
<td>Finland</td>
<td>4</td>
<td>IPT</td>
<td>180</td>
<td>85</td>
<td>µmol N m⁻² d⁻¹</td>
<td>Rissinan et al. (2013)</td>
</tr>
<tr>
<td>Wucheng</td>
<td>China</td>
<td>6</td>
<td>MIMS</td>
<td>47</td>
<td>54</td>
<td>mg N m⁻² h⁻¹</td>
<td>Wang et al. (2017)</td>
</tr>
<tr>
<td>Yunan-Guizhou</td>
<td>China</td>
<td>20</td>
<td>C₂H₂</td>
<td>41</td>
<td>1.06</td>
<td>ng N g⁻¹ h⁻¹</td>
<td>Liu et al. (2015)</td>
</tr>
</tbody>
</table>

2001) did not detect significant spatial differences in denitrification, despite strong spatial gradients in benthic nutrient fluxes, organic carbon, salinity and nitrogen content. In fact, different studies suggest different sets of controlling factors at different spatial scales (Rissanan et al., 2011; Palta et al., 2014; this study). This makes spatial denitrification studies very interesting, albeit inconclusive regarding controlling factors. Clearly, more studies are needed.

Comparison of denitrification rates across different freshwater districts and within a single environment resulted in high ranges of variability among (CVs: 15-119 %) and within (CVs: 6-139 %) (Tables 2-3). A naïve idea would be to suggest that larger area ranges would result in higher rate variabilities and thus CVs at the single-lake scale could be lower than CVs at the district scale because higher environmental variability implies higher variability of denitrification rates. However, a preliminary meta-analysis of compiled data in Tables 2-3 does not support such a hypothesis because variabilities of both groups were not statistically different (p = 0.67; Mann-Whitney test). Rissanan et al. (2013) report that rate variabilities at both spatial scales are similar, but they can differ when those measurements are undertaken at the continental scale. This highlights the notion of “hotspots” (see below; Groffman et al., 2009), which are sites where micro-environmental conditions enhance denitrification rates from those recorded in neighbour sites.

All in all, our results point to preferential sites of denitrification in a chain of lakes and on the single-lake scale as well. The importance of these hotspots for denitrification has also been suggested by Brusewitz et al. (2012) for Gull Lake. While the reason for this is obvious (joint conditions of several controlling factors enhancing rates), it is very difficult to gain short-scale knowledge of them prior to N process incubations, such as those needed for assessing denitrification when using the incubation approach. Therefore, to enhance our knowledge of denitrification at different spatial scales more efforts must be devoted to modelling (Groffman et al., 2009). Thus, irrespective of the difficulties arising from the choice of a given methodology for denitrification assessment (Seitzinger et al., 1993; Groffman et al., 2006), the most problematic issue when dealing with this process appears to be the uncertainty in spatial rates, which is very hard to cope with.

CONCLUDING REMARKS

Our finding of high spatial intra-variability of denitrification in a single lake may be especially relevant to sampling strategies where spatial and longitudinal studies of denitrification could provide more accurate data of the process. Inter- and intra-lake variability could be driven by factors that show temporal and spatial variability and that could also be acting at both regional- and local scales. All this would broaden our vision and give a more integrated view of current knowl-
edge of the denitrification process in lakes. This issue is of great environmental importance in nitrate-polluted lakes like those of the Ruidera complex and others lying in agricultural catchments. However, to accurately tackle the spatial vagaries of the process is highly challenging and hence modelling could be a much cheaper solution to ascertain spatial variability in denitrification rates, at least in freshwaters.

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