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DEPARTAMENTO DE ECOLOGÍA E HIDROLOGÍA

Mediterranean wetland functionality in agricultural landscapes. Driving factors for spatial and temporal variability in N and P retention

Funcionalidad de los humedales mediterráneos en paisajes agrícolas. Factores responsables de la variabilidad espacial y temporal de la retención de N y P.

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Directora: Rosa María Gómez Cerezo

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Resumen

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La contaminación difusa de nutrientes procedente de la agricultura es un grave problema en la gestión del agua. En las últimas décadas, medidas de gestión como la creación de humedales artificiales y la utilización de humedales naturales han tenido un importante papel en la reducción de la contaminación difusa, mediante la retirada de nutrientes del agua de escorrentía agrícola. Sin embargo, la diversidad de procesos implicados en la retención de nutrientes determina que la eficiencia de retención de los humedales pueda variar en función de las condiciones climáticas y los regímenes hidrológicos. En este sentido, la funcionalidad de los humedales mediterráneos del sureste ibérico para la retención de nutrientes en paisajes agrícolas es en gran medida desconocida.

Introducción general

El problema de la contaminación difusa en cuencas agrícolas

El papel fundamental de los ecosistemas acuáticos continentales en la biosfera es el suministro de agua y su conducción desde los continentes hasta el mar (Bailey et al. 2004). No obstante, los sistemas fluviales no sólo actúan como medios receptores, sino que tienen la facultad de transformar, retener y eliminar parte de los nutrientes que transportan en el agua, influyendo con ello en su calidad (Peterson et al. 2001; Alexander et al. 2009). Además, sustentan comunidades ecológicas únicas y complejas, y a menudo definen la estructura y el funcionamiento de los ecosistemas terrestres circundantes (Bailey et al. 2004). Por ello, y debido a su papel relevante en el suministro de agua potable, durante las últimas décadas ha habido un creciente interés por desarrollar estrategias de gestión que reduzcan los impactos de la actividad humana sobre los ecosistemas acuáticos (ej. el proyecto europeo NICOLAS (Nitrogen Control by Landscape Structures in agricultural environments - 1998); la Directiva Marco del Agua (2000/60/EC); el programa CWSRF (Clean Water State Revolving Fund-USEPA- 2009)).

Actualmente, la agricultura intensiva está globalmente reconocida como una fuente importante de contaminación difusa, que contribuye significativamente al incremento de la concentración de nitrógeno (N) y fósforo (P) en los ecosistemas acuáticos continentales (Mason et al. 1990; Carpenter et al. 1998a; Correll 1998; Baker y Richards 2003; Nord y Lanyon 2003; Conley et al. 2009; Kopacek et al. 2013). Esta contaminación es más difícil de controlar que la contaminación puntual, aunque puede reducirse mediante técnicas de gestión adecuadas, como por ejemplo, mediante la disminución del aporte de fertilizantes agrícolas, la reducción de la escorrentía agrícola o la conservación del suelo.

El enriquecimiento excesivo en N y P de las aguas continentales puede resultar en serios problemas de eutrofización, con consecuencias ambientales tales como el incremento de la abundancia de algas y plantas acuáticas, hipoxia, mortandad de peces y pérdida de biodiversidad (Vitousek et al. 1997; Smith 2003) así como, con consecuencias para la salud humana (Townsed et al. 2003). Además, la eutrofización puede tener también consecuencias económicas negativas, ya que puede causar una pérdida de los servicios que los ecosistemas acuáticos proveen, como son el suministro de agua para abastecimiento, uso industrial y agrícola, o el uso recreativo (Carpenter et al. 1998b).

En la región mediterránea, y especialmente en las zonas más áridas como el sureste ibérico, el agua ha sido uno de los factores limitantes de la actividad económica (Prat y Munné 2000). No obstante, el trasvase de agua entre cuencas ha permitido que los cultivos de regadío ocupen grandes extensiones de terreno y ejerzan una intensa presión sobre los ecosistemas acuáticos continentales, entre otros problemas ambientales (Martínez-Fernández et al. 2000; Esteve 2003; Martínez-Fernández y

Esteve 2006). De hecho, los aportes de los sobrantes de riego y los lixiviados de nutrientes y otros contaminantes provenientes de los cultivos generan importantes problemas de calidad del agua (Gómez et al. 2005).

Este problema es especialmente acusado en las regiones áridas y semiáridas, donde la mayoría de los cursos de agua presentan escasos caudales que, entre otros efectos, disminuyen su capacidad de dilución de solutos y los vuelve especialmente sensibles a la eutrofización (ej. Álvarez-Cobelas et al. 2005; Arce et al. 2013). En este contexto, la intercepción y disminución de la contaminación difusa es un requisito indispensable para prevenir y gestionar la eutrofización de los ecosistemas acuáticos en general, y de las cuencas agrícolas del sureste ibérico en particular.

Los humedales

Los humedales son un rasgo común de muchos paisajes en todo el mundo. Su estudio como ecosistema comenzó en la década de los 40 (Lindeman 1942; Odum, 1957) y aunque desde entonces son muchas las definiciones que se han dado del término “humedal” (ej. Cowardin et al. 1979; Howard-Williams 1985; Ramsar-Bureau 1988), en todas ellas el agua es siempre el factor dominante que determina su presencia.

En términos generales, los humedales son áreas donde el nivel freático está cerca o sobre la superficie del suelo, lo que determina el desarrollo del mismo y de las comunidades de animales y plantas que viven allí (Cowardin et al. 1979). Comparten propiedades de los sistemas acuáticos y terrestres, y de los sistemas lóticos y leníticos, constituyendo importantes zonas ecotono, con elevados valores de biodiversidad, que desempeñan también un importante papel como reguladores metabólicos de ambos sistemas (Howard-Williams 1985; Wetzel 1990). Los humedales constituyen especialmente los hábitats de las aves acuáticas, es decir, son las zonas de marisma, pantanos, turberas o aguas rasas, naturales o artificiales, permanentes o temporales, de aguas remansadas o corrientes, dulces, salobres o salinas, con inclusión de aguas marinas cuya profundidad en marea baja no exceda de los seis metros (Ramsar-Bureau 1988). En 1988, González-Bernáldez adecuó el término “humedal” para su utilización en zonas áridas y semiáridas, y lo definió como aquellas formaciones que, sin ser ríos o lagos, representan una anomalía hídrica positiva en el paisaje, bien sea temporal o espacial.

En las zonas áridas y semiáridas, las escasas precipitaciones (con alternancia de lluvias torrenciales de corta duración y largos periodos de sequía), la elevada evapotranspiración (mayor que la precipitación durante la mayoría de los meses) y los infrecuentes eventos de escorrentía y recarga de agua subterránea (Gasith y Resh 1999), determinan que los humedales sean sistemas singulares dentro del paisaje (González-Bernáldez 1989; Hollis 1990)

Se trata generalmente de zonas de descarga de agua subsuperficial procedente de la propia cuenca (Hollis 1990) que, desde un punto de vista funcional, regulan el microclima generando un entorno más húmedo y fresco (González-Bernáldez y Pérez 1988), y juegan un papel determinante en la retención de sedimentos y contaminantes, así como, en la dinámica de nutrientes a escala de cuenca (Gómez 1995).

Aunque estos humedales suelen ocupar pequeñas superficies de terreno, son sistemas con una alta productividad biológica (Hollis 1990), ya que permanecen activos en el verano, cuando el resto del territorio sufre un importante déficit hídrico. En este sentido, son anomalías biogeográficas, ya que son el refugio de muchas especies vegetales y animales (González-Bernáldez et al. 1987; González-Bernáldez 1989).

En general, el clima y la geología determinan la fenología y el funcionamiento de los humedales (Alonso et al. 1985). En el sureste ibérico, la escasez de precipitaciones y la intensa evaporación propia del clima mediterráneo, junto con la abundancia de suelos margosos fácilmente erosionables y ricos en sales, determina la existencia de humedales que presentan un régimen hídrico temporal y una elevada salinidad, que a su vez determina la existencia de comunidades vegetales halófilas. Estos humedales se localizan en las zonas costeras (marismas litorales), en la parte más deprimida de extensas llanuras aluviales o asociados a sistemas de drenaje (Ramirez-Díaz 1992; Gómez 1995; Ballester 2003a; Ballester 2003b).

Los humedales asociados a sistemas de drenaje, de acuerdo con su localización en el paisaje y con su hidrología, son denominados en la literatura anglosajona bajo el término “slope wetlands” (Stein et al. 2004; Acreman 2005) (Fig. 1). En el sureste ibérico, estos humedales se desarrollan en el lecho de pequeñas vaguadas o ramblas que terminan desembocando en cauces de mayor orden (ramblas, ríos o arroyos). Concretamente se localizan en zonas donde se dan las condiciones topográficas necesarias que permiten al agua subsuperficial, procedente de la parte alta de la cuenca, alcanzar la superficie del suelo y crear una zona de humedad más o menos permanente (Fig. 1). Este aporte de agua subsuperficial constituye la principal vía de entrada de agua y nutrientes en estos humedales (Gómez 1995; Gómez et al. 2001), y tiene lugar en la cabecera de los mismos, donde abundan los helófitos, fundamentalmente *Phragmites australis*. Además, reciben de forma más esporádica, a modo de pulsos, aportes directos de agua de la precipitación y de las avenidas, si estas tienen lugar.

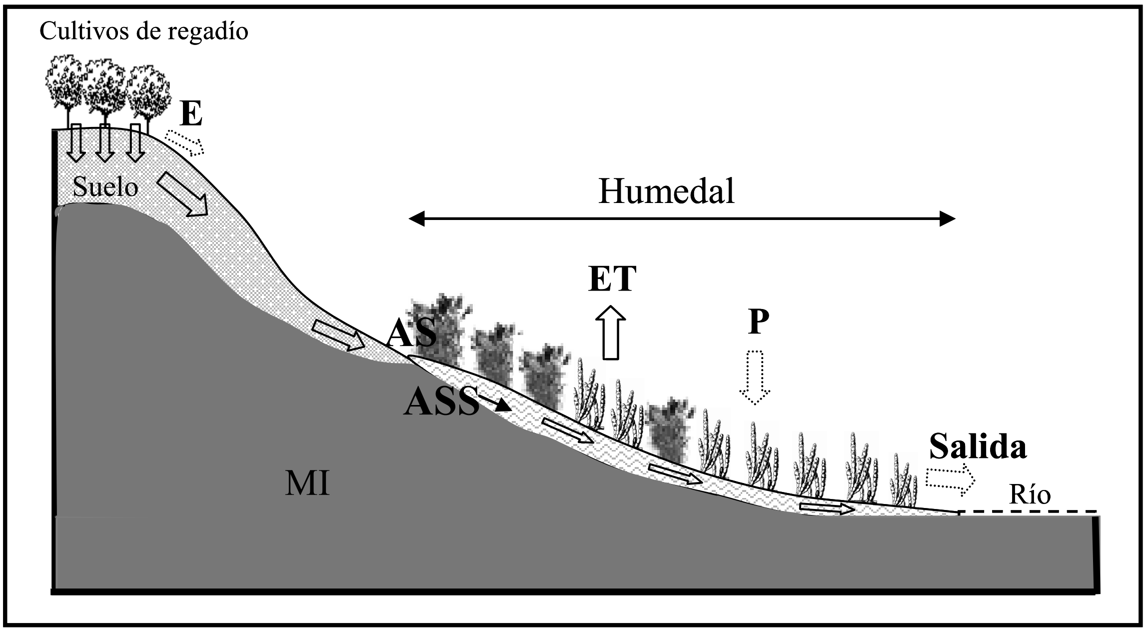

Fig. 1. Vista de la sección vertical de un humedal asociado a sistemasde drenaje "slope wetland" en la que se muestra las principales vías de entrada y salida de agua, así como los diferentes compartimentos. E: agua de escorrentía; AS: agua superficial; ASS: agua subsuperficial; MI: material geológico impermeable; ET: evapotranspiración y P: precipitación. El grosor de línea de las flechas hace referencia a la magnitud de la entrada/salida.

El agua, una vez en el humedal, fluye a favor de la pendiente como una lámina somera entre la vegetación halófila, dominada por *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa* y *Suaeda vera*, que sustituye al carrizal conforme la salinidad del agua aumenta (Fig. 1). También suelen aparecer pequeñas manchas dispersas de *Tamarix canariensis*, *Tamarix boveana* y *Juncus maritimus*. Entre los pies de la vegetación emergente, sobre los sedimentos, es común la presencia de tapices de biofilm (matriz polisacarídica constituida por una comunidad compleja de bacterias, algas, hongos y protozoos) así como, la presencia de los macrófitos *Vaucheria dichotoma y Ruppia marítima,* este último de forma más reducida (Gómez 1995). En ocasiones, el agua subsuperficial que da origen al humedal no llega nunca a aflorar en superficie, de forma que estos humedales sólo presentan flujo subsuperficial. Sin embargo, tanto los helófitos como la vegetación halófila descrita siguen estando presentes.

En cuanto a la dinámica temporal, la sequía y las avenidas constituyen las perturbaciones naturales a las que están sometidos estos sistemas (Gómez 1995; Gómez et al. 1995). Durante el estiaje, el descenso del nivel freático y la elevada evapotranspiración ocasionan frecuentemente la desaparición de la lámina de agua superficial. En el otoño, si se producen fuertes precipitaciones, pueden ocurrir avenidas que provocan la entrada de agua, nutrientes, materia orgánica y sedimentos, así como, la movilización de los materiales del lecho.

Por último, cabe destacar que a pesar de que estos humedales son elementos singulares dentro del paisaje Mediterráneo y en especial, en el contexto europeo, pues reúnen hasta 4 tipos de hábitats de interés comunitario (pastizales salinos mediterráneos (*Juncetalia maritim)*; matorrales salinos mediterráneos y termo-atlánticos (*Sarcocornetea fruticosi*); matorrales halonitrófilos (*Pegano-Salsoletea*) y estepas salinas mediterráneas (*Limonietalia*)), están sometidos a una fuerte presión antrópica (Vidal-Abarca et al. 2000; Gómez et al. 2005). Su localización, en cuencas sedimentarias con un importante porcentaje de suelo dedicado a la agricultura, sus reducidas dimensiones y su nulo reconocimiento social como ecosistemas singulares propician que sufran multitud de impactos con total impunidad, entre los que destacan el vertido de basura y escombros, el tráfico rodado y la desecación y/o roturación para su puesta en cultivo (Gómez et al. 2005).

Desde la esquina izquierda superior a la derecha inferior: imagen del ortofoto de la Región de Murcia (2002) donde se observa un mosaico de cultivos de regadío y su localización con respecto a la red de drenaje; vista panorámica del humedal de la Parra donde se aprecia el cambio de comunidades vegetales, de helófitos a halófitos; vista del tramo bajo del humedal del Taray donde se aprecia el predominio de los halófitos; tramo medio del humedal del Taray; lámina de agua superficial, biofilm y hojarasca entre los pies de *Pragmites australis* del transecto 1 del humedal del Taray; piezómetros en el transecto 3 del humedal de la Parra.

Importancia de los humedales en el control de la contaminación difusa

Los humedales no son sólo ecosistemas de gran importancia por sus valores intrínsecos, sino además por los servicios ecosistémicos que proveen. Entre estos, hay que mencionar la importancia de los procesos biogeoquímicos que en ellos tienen lugar y que determinan su importante función como filtros naturales de nutrientes y otros contaminantes (ej. Mitsch y Gosselink 2000; Fisher y Acreman 2004; Verhoeven et al. 2006).

En términos generales, está globalmente reconocido que tanto los humedales naturales, donde quedan incluidos los bosques de ribera, como los artificiales reducen el flujo de nutrientes desde los ecosistemas terrestres a los acuáticos, siendo ambos utilizados en el tratamiento de la contaminación difusa en zonas agrícolas, urbanas e incluso mineras (Lowrance et al. 1984; Mitsch 1992; Mitsch et al. 2000; Braskerud 2002a; Braskerud 2002b; Álvarez-Rogel et al. 2006; Knox et al. 2008; Vymazal y Kröpfelová 2008; Ardon et al. 2010). En este contexto, la Directiva Marco del Agua (2000/60/EC) destaca el papel de los humedales naturales como elementos necesarios dentro de las redes de drenaje para obtener el buen estado ecológico de las aguas superficiales y subterráneas. No obstante, debemos evitar considerar a los humedales naturales como meras “plantas de depuración de agua”.

Los principales procesos biológicos responsables de la retirada de nutrientes en los humedales son la asimilación por la vegetación y los microorganismos, que es una retirada temporal de N y P, y la desnitrificación, proceso microbiológico que supone una retirada permanente de N del medio (ej. Reddy and Patrick 1984; Groffman et al.

1992). Otro proceso directamente implicado en la disminución de la concentración de P es su retención en los sedimentos, siendo en ocasiones éste el principal mecanismo de retirada (ej. González-Alcaraz et al. 2012). En general, la efectividad de todos estos procesos depende del tipo de vegetación, las condiciones fisicoquímicas del suelo, la temperatura del agua, la disponibilidad de carbono orgánico y $N\text{-}NO_3^-$, la profundidad de la tabla de agua o la velocidad de la corriente (Howard-Williams 1985; Bowden 1987; Reddy et al. 1995; Pinay et al. 2007).

En el sureste ibérico, las condiciones climatológicas confieren a los humedales gran potencial para ser considerados elementos eficaces en el control de la contaminación difusa. La elevada radiación solar y la temperatura suave a lo largo del año facilitan que los procesos biológicos implicados en la transformación y eliminación de nutrientes puedan permanecer activos durante más tiempo, en comparación con humedales de zonas más templadas (ej. Spieles and Mitsch 2000; Pinay et al. 2007). Por otra parte, su estratégica localización en los paisajes agrícolas les permite interceptar los flujos de agua superficial y subsuperficial, y actuar sobre su carga en nutrientes, antes de que alcancen otros sistemas acuáticos. Por ejemplo, estudios previos realizados en marismas costeras (Álvarez-Rogel et al. 2006; González-Alcaraz et al. 2012) han constatado la eficacia de estos humedales reduciendo las concentraciones de N y P. Sin embargo, se desconoce el papel que los humedales de interior asociados a redes de drenaje pueden desempeñar en el control de la contaminación difusa, dado su menor tamaño y las condiciones específicas que los caracterizan.

El estudio de dicha capacidad no solo permite analizar la utilidad de estos humedales como herramienta de control de la contaminación difusa, mejorando con ello la calidad del agua de los ecosistemas acuáticos, sino que pone en valor la conservación de estos sistemas. De hecho, la conservación y/o restauración de los humedales de interior en paisajes eminentemente agrícolas, donde las diferentes actividades humanas compiten por el suelo, sólo es posible mediante la puesta en valor y el reconocimiento social de los servicios que éstos ofrecen.

Objetivos y estructura de la presente memoria

La presente memoria pretende aportar información sobre distintos aspectos relacionados con la capacidad de los humedales asociados a sistemas de drenaje del sureste ibérico para retirar N y P del agua. Dicha información será de utilidad para conocer el papel que desempeñan en el control de la contaminación difusa en cuencas agrícolas y con ello, en la mejora de la calidad del agua, lo que podrá suponer su reconocimiento y revalorización como sistema natural. En este contexto se plantean cuatro objetivos generales:

- Conocer la eficiencia de retención de N y P de los humedales asociados a sistemas de drenaje, así como, su variación temporal a lo largo de un ciclo hidrológico.

- Conocer si existen diferencias en la eficiencia de retención de N y P entre el compartimento superficial y subsuperficial de estos humedales.

- Analizar los factores ambientales que afectan a la retención de N y P en estos sistemas.

- Analizar la variabilidad espacial, a lo largo del eje longitudinal de los humedales, de la eficiencia de retención de N y P en ambos compartimentos.

En base a los objetivos mencionados, la presente memoria queda estructurada en tres capítulos que se han escrito como publicaciones independientes y cuyos objetivos específicos se describen a continuación:

Capítulo 1

Los objetivos de este capítulo son 1) cuantificar la eficiencia de retención de las diferentes fracciones del N (N-nitrato (N-NO_3^-), N-amonio (N-NH_4^+), N-nitrógeno orgánico total (N-NOT) y nitrógeno total (N-NT)) en el compartimento superficial de dos humedales mediterráneos afectados por escorrentía agrícola, 2) analizar la variabilidad temporal de la eficiencia de retención de las diferentes fracciones de N a lo largo de un ciclo hidrológico, y 3) examinar el efecto de la hidrología y la carga de N sobre dicha eficiencia de retención.

El contenido de este capítulo ha sido publicado en el siguiente artículo:

García-García V, Gómez R, Vidal-Abarca MR, Suárez ML (2009) Nitrogen retention in natural Mediterranean wetland-streams affected by agricultural runoff. Hydrology and Earth System Sciences 13: 2359-2371

Capítulo 2

Los objetivos de este capítulo son 1) cuantificar la eficiencia de retención, durante un ciclo hidrológico, de las diferentes fracciones del N (N-NO_3^-, N-NH_4^+, N-NOT y N-NT) en el compartimento subsuperficial de dos humedales mediterráneos afectados por escorrentía agrícola, 2) examinar el efecto de la hidrología y la carga de N sobre dicha eficiencia de retención, y 3) comparar la retención de N entre el compartimento superficial y el subsuperficial, y establecer si existen diferencias.

El contenido de este capítulo ha sido publicado en el siguiente artículo:

García-García V, Gómez R, Vidal-Abarca MR, Suárez ML (2013) Subsurface N retention in two Mediterranean wetland-streams affected by agricultural runoff. Wetlands 33: 597-608

Capítulo 3

El objetivo de este capítulo es 1) examinar la variabilidad espacial, dentro del eje longitudinal de los humedales, de la eficiencia de retención de las distintas fracciones del N (N-NO_3^-, N-NH_4^+, N-NOT y N-NT) y del P (fósforo reactivo soluble (PRS), fósforo particulado (P-Part) y fósforo total (PT)) en el compartimento superficial y subsuperficial de dos humedales mediterráneos afectados por escorrentía agrícola.

Este capítulo se corresponde con el artículo enviado para su revisión y posterior publicación:

García-García V, Gómez R (enviado) Spatial variability in N and P retention through reaches in two Mediterranean slope wetlands. Ecological engineering

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Capítulo 1. Retención de nitrógeno (N) en humedales mediterráneos asociados a sistemas de drenaje y afectados por escorrentía agrícola

Resumen

En muchas regiones del mundo, la contaminación difusa procedente de la agricultura ha sido reconocida como la principal fuente antrópica de N para los ecosistemas acuáticos. En este sentido, numerosos estudios evidencian la capacidad que tienen los humedales para controlar la contaminación difusa de N a escala de cuenca, aunque la variabilidad de sus características biológicas, hidrológicas y físico-químicas hace difícil predecir la eficiencia de retención de los diferentes tipos de humedales. De acuerdo con ello, en el presente capítulo se cuantificó la eficiencia de retención de N en el compartimento superficial de dos humedales Mediterráneos asociados a sistemas de drenaje y afectados por escorrentía agrícola. Además, se analizó la variabilidad temporal de la eficiencia de retención de N a lo largo de un ciclo hidrológico y se examinó el efecto de la hidrología y la carga de N sobre dicha eficiencia.

Para ello, se analizó la concentración de N-NO_3^-, N-NH_4^+, N-NT, N-NOT y cloruros (Cl^-) en el agua superficial de ambos humedales. Las muestras de agua fueron recogidas mensualmente, a lo largo de un ciclo hidrológico, en cuatro transectos que fueron establecidos perpendicularmente a la dirección del flujo y separados entre sí por 100 m.

El análisis de las eficiencias de retención mostró que ambos humedales redujeron consistentemente la concentración de N. La mayor eficiencia de retención fue para el N-NO_3^- (72.3%), a pesar de la elevada concentración media de entrada (> 20 mg l^{-1} en ambos humedales). Los humedales también retuvieron N-NOT (8.4%) pero sin

embargo, exportaron N-NH_4^+. A lo largo del periodo de estudio, los humedales del Taray y la Parra retiraron un valor medio de 1.6 y 0.8 kg N-NO_3^- día^{-1}.

La eficiencia de retención de N-NO_3^- siguió un patrón estacional, registrándose los valores más elevados en verano (Junio-Septiembre). La variabilidad temporal de las eficiencias de retención de N-NOT y N-NH_4^+ fue considerablemente mayor que la del N-NO_3^- y no mostró un patrón estacional. El análisis de regresión lineal múltiple mostró que el 81.5% de la variabilidad temporal de la eficiencia de retención de N-NO_3^- fue explicada por la retención hidrológica, que afectó de forma positiva, y por la concentración de entrada de N-NO_3^-, que lo hizo de forma negativa ($R^2_{adj}=0.815$, $p<0.01$, n=25). En el caso del N-NOT y el N-NH_4^+ no se obtuvieron modelos de regresión estadísticamente significativos.

Éstos resultados muestran cómo la conservación de estos pequeños humedales mediterráneos, tan ampliamente distribuidos y estratégicamente localizados en los tramos altos de cuencas sedimentarias del sureste ibérico, puede ayudar no sólo a mejorar la calidad del agua en las cuencas agrícolas, sino también a alcanzar el buen estado ecológico de las aguas superficiales, objetivo final de la Directiva Marco del Agua.

Capítulo 2. Retención subsuperficial de nitrógeno (N) en dos humedales Mediterráneos asociados a sistemas de drenaje y afectados por escorrentía agrícola

Resumen

En las cuencas sedimentarias del sureste ibérico es frecuente la existencia de pequeños humedales asociados a los sistemas de drenaje. Éstos interceptan los flujos de agua superficial y subsuperficial generados dentro de dichas cuencas, la mayoría de las cuales tienen como principal uso del suelo la agricultura de regadío, lo que implica que el agua que reciben estos sistemas presente generalmente una elevada concentración de N.

Este tipo de humedales tiene una elevada capacidad para retener N en el compartimento superficial. Sin embargo, muchos de ellos no presentan lámina de agua superficial, y todo el agua de escorrentía que reciben fluye a través del compartimento subsuperficial. De acuerdo con ello, y considerando además que existen diferencias en la estructura física y en las características químicas y biológicas de ambos compartimentos, se establece la necesidad de conocer la eficiencia de retención de N en el compartimento subsuperficial, para así poder determinar el papel que tienen estos humedales en el control de la contaminación difusa a escala de cuenca.

Los objetivos de este capítulo fueron cuantificar la eficiencia de retención de N en el compartimento subsuperficial de dos humedales asociados a sistemas de drenaje y afectados por escorrentía agrícola, y analizar la influencia de la hidrología y la carga de N sobre dicha eficiencia. Además, se establecen si existen diferencias en la eficiencia de

retención de N entre el compartimento subsuperficial y el superficial, analizado en el primer capítulo.

La eficiencia de retención de N subsuperficial fue elevada y mayor que la retención observada en el compartimento superficial. Los humedales claramente retuvieron N-NO_3^- (96.5% y 89.8%), la fracción dominante del N. Además, generalmente retuvieron N-NOT (49.9% y 21.6%), pero sin embargo, retuvieron o exportaron N-NH_4^+ dependiendo del humedal.

La temperatura del agua explicó el 59% y el 34% de la varianza de la eficiencia de retención de N-NO_3^-. Este resultado fue consistente con estudios previos y subraya el importante papel de los procesos biológicos en la retención de N-NO_3^-. No obstante, esta relación positiva entre ambas variables pudo ser también consecuencia de los mayores tiempos de residencia del agua durante los meses de verano, cuando se alcanzan las mayores temperaturas del agua.

Los humedales de estudio no mostraron “síntomas de saturación” a elevadas concentraciones de N-NO_3^- (40 mg l^{-1}). De hecho, pueden ser comparados con los bosques de ribera en términos de su alta eficiencia de retención de N-NO_3^- subsuperficial. Sin embargo, debido a los bajos caudales subsuperficiales, la contribución del compartimento subsuperficial al balance global de retención de N-NT en el humedal fue menor (0.01% y 0.05%) que la del compartimento superficial (83.1% y 39.2%). Los humedales retuvieron una media de 0.028 y 0.15 mg N-NT m^{-2} $día^{-1}$ en el compartimento subsuperficial.

Capítulo 3. Variabilidad espacial en la retención de N y P a través de tramos en dos humedales mediterráneos asociados a sistemas de drenaje

Resumen

Es ampliamente conocido que los humedales pueden significativamente disminuir la concentración de N y P en el agua de escorrentía agrícola, mejorando la calidad del agua de los ecosistemas acuáticos localizados aguas abajo de ellos. No obstante, algunas cuestiones permanecen sobre la función tampón de los humedales: ¿la retención de nutrientes es uniforme a lo largo del humedal? o ¿hay determinadas zonas de humedal más eficientes que otras?

En áreas bajo una fuerte presión de utilización, la conservación de los humedales entra en conflicto con los usos del suelo. En este sentido, una eficiente planificación del uso del suelo con una gestión de la calidad del agua integrada demanda la selección de las zonas de humedal más adecuadas y posteriormente, el dimensionamiento del humedal como sistema tampón a escala de cuenca.

Así, el principal objetivo de este estudio fue analizar la variabilidad espacial en la retención de N y P a través de tramos en dos humedales mediterráneos (Taray y Parra) asociados a sistemas de drenaje de cuencas agrícolas. Los resultados mostraron que a la escala espacial de humedal, el PT y el PRS fueron retenidos en el Taray, pero exportados en la Parra. No obstante, la cantidad de PT retenida en el Taray (0.03 g m^{-2} y^{-1}) fue baja en comparación con las cantidades retenidas por otros humedales de pequeñas dimensiones. Sin embargo, el análisis a escala espacial de tramo mostró que la tasa de retención (mg m^{-1} dia^{-1}) no tuvo un carácter lineal. Así, los primeros 100 m de

los humedales, los tramos más altos, fueron significativamente más efectivos retirando NO_3^- y NT en masa de los compartimentos superficial y subsuperficial que los tramos más bajos (200 y 300 m). Aunque las diferencias entre tramos en la retención en masa de P no fueron estadísticamente significativas, se observó una tendencia a retener PT y PRS a lo largo de los primeros 100 m, y a exportar en los tramos más bajos. La mayor retención en masa de N y P en el primer tramo pudo estar principalmente relacionada con la asimilación por *Phragmites australis* y la incorporación a su biomasa, la retirada de P a través de la formación de placas de Fe en la superficie de sus raíces, y con la desnitrificación.

Finalmente, los resultados muestran que un humedal asociado a sistemas de drenaje con una longitud de 100 m podría retirar una cantidad media de 162-255 kg NO_3^- y^{-1} y 174-282 kg NT y^{-1}. En base a las magnitudes de los flujos de N y P estimados en paisajes agrícolas de clima templado, la preservación de un 10% de área agrícola para la conservación de un humedal, podría retirar la cantidad de N exportada de paisajes agrícolas manejados tanto intensivamente como extensivamente.

Conclusiones generales

1. Los humedales de estudio mostraron altas eficiencias de retención de N, tanto en el compartimento superficial (87.9% en el Taray y 50.9% en la Parra) como en el subsuperficial (91.3% en el Taray y 82.2% en la Parra), incluso frente a elevadas concentraciones de N (27.2 y 27.5 mg l^{-1} y 17.7 y 50.4 mg l^{-1} para los humedales Taray y Parra, respectivamente). Los bajos caudales, la elevada temperatura del agua, e indirectamente, el prolongado tiempo de residencia del agua en el humedal favorecieron posiblemente la actuación de los procesos biológicos implicados en la retención de N, a lo largo de todo el año. Así, el clima cálido parece constituir una ventaja para la retención de N en los humedales mediterráneos, frente a los humedales de climas templados.

2. El NO_3^- fue la fracción mayoritaria del N en ambos compartimentos de los humedales. Además, fue la forma del N que mayores porcentajes de retención mostró en ambos humedales, con valores medios de 72.3% en el compartimento superficial y 93.2% en el subsuperficial. La variabilidad de la eficiencia de retención de NO_3^- a lo largo del periodo de estudio, fue menor en el compartimento subsuperficial que en el superficial.

3. En el compartimento superficial, la hidrología y la concentración de entrada de NO_3^- fueron los principales factores ambientales que afectaron a la eficiencia de retención del NO_3^-. En el compartimento subsuperficial, la temperatura del agua fue el único factor que explicó parte de la varianza observada en la eficiencia de retención de NO_3^-.

4. En general, las mayores eficiencias de retención de NO_3^- se dieron en los meses de verano, coincidiendo con el incremento de la temperatura del agua y el descenso del caudal superficial en los humedales.

5. En ambos compartimentos, las eficiencias de retención de NH_4^+ y de NOT fueron muy inferiores y más variables a lo largo del periodo de estudio que la eficiencia de retención de NO_3^-. En determinadas ocasiones, ambos humedales exportaron NH_4^+ y NOT. La propia variabilidad de las condiciones locales (a nivel de microhábitat) del potencial redox y del contenido en materia orgánica de los sedimentos, podría explicar los casos de exportación de N. Ningún factor ambiental de los estudiados explicó la variabilidad temporal de la eficiencia de retención del NH_4^+ y del NOT.

6. A pesar de que el compartimento subsuperficial mostró una mayor eficiencia de retención de NO_3^- que el superficial, el escaso caudal subsuperficial de los humedales determinó que la carga de NO_3^- retirada en este compartimento (0.09 ± 0.08 mg m^{-2} $día^{-1}$, n=25) fuese muy inferior a la retirada en el compartimento superficial (215.4 ± 217.7 mg m^{-2} $día^{-1}$, n=25).

7. A pesar de las bajas concentraciones de P en el agua de entrada a los humedales, a escala global, mientras que el humedal del Taray retuvo P en sus distintas formas (PRS, P-Part y PT), el humedal de la Parra lo exportó. En cualquier caso, la cantidad de PT retenido por superficie en el humedal del Taray fue baja (0.03 g m^{-2} y^{-1}), en comparación con otros humedales (restaurados o construidos) en zonas agrícolas.

8. La eficiencia de retención de N y P en los humedales estudiados no mostró un carácter lineal, es decir, el incremento en la longitud del humedal, no generó un incremento proporcional en la retención de nutrientes.

9 La mayor eficiencia de retención de NT y NO_3^- (99.9% del NT) se observó en los primeros 100 m de humedal (tramo alto). En este tramo, la retención media de NO_3^- de ambos humedales fue 41.2% en el compartimento superficial y 64.9% en el compartimento subsuperficial.

10. A pesar de que las diferencias entre tramos no fueron estadísticamente significativas, la eficiencia de retención de NH_4^+ en el compartimento superficial fue mayor en el último tramo del humedal, donde las altas concentraciones de NH_4^+ pudieron estimular su asimilación microbiológica. La eficiencia de retención del NOT no mostró ningún patrón de variación espacial consistente entre tramos.

11. A pesar de que las diferencias en la cantidad de PT and PRS retenido entre tramos no fueron estadísticamente significativas, en general el PRS fue retenido en los primeros 100 m de la superficie del humedal (oscilando entre 11.8 ± 22.5% y 22.02 ± 5.8%), siendo exportado en el resto de tramos. La disminución del potencial redox de los sedimentos en los tramos bajos del humedal, podría explicar su incremento en el agua superficial de esta zona del humedal.

12. La presencia y elevada biomasa de *Phragmites australis* en los tramos altos de ambos humedales podría estar relacionada con la mayor eficiencia de retención de N y P en ellos.

13. Nuestros resultados muestran que 100 m lineales de un humedal asociado a sistemas de drenaje (”slope-wetland”) pueden llegar a retirar por término medio entre 162-255 kg NO_3^- $año^{-1}$ y 174-282 kg TN $año^{-1}$. Considerando un transporte global de N (es decir, de N lixiviado y en escorrentía superficial) desde las zonas de cultivo que puede oscilar entre 15-75 kg N $ha^{-1}año^{-1}$, si se trata de zonas de agricultura intensiva, o

entre 1.5-19 kg N $ha^{-1}año^{-1}$, si son zonas de agricultura extensiva, el destino de un 10% de la superficie agrícola a la conservación del humedal, podría garantizar la retirada de la carga de N exportada por los cultivos y la disminución del flujo de P de un 11.8 ± 22.5%, a un 22.02 ± 5.8% .

14. La protección y/o restauración de los humedales asociados a sistemas de drenaje, podría ser una herramienta muy apropiada para controlar la contaminación difusa en cuencas agrícolas. En este sentido, una estrategia útil para promover su protección sería destacar su importante función en los paisajes agrícolas. Sin embargo no hay que olvidar que la primera medida a llevar a cabo para mejorar la calidad del agua es la de disminuir el uso de fertilizantes o la reducción de la escorrentía agrícola. De no ser así, la elevada carga de nutrientes que estos sistemas podrían llegar a recibir puede ocasionar a largo plazo un “efecto de saturación”, lo cual resultaría en indeseables efectos ambientales tanto para el humedal, como para los ecosistemas acuáticos situados aguas abajo.

General introduction

General introduction

Nonpoint source pollution of nutrients from agriculture areas is a major problem in water management. In the last decades, management measures such as the creation of constructed wetlands and the utilisation of natural wetlands have had an important role in the reduction of diffuse pollution, by removing nutrients from agricultural runoff. However, the diversity of processes involved in nutrient retention determines that retention efficiency in wetlands can vary in function of climatic conditions and hydrological regimes. In this sense, the functionality of Mediterranean wetlands in the Iberian southeast for nutrient retention in agricultural landscapes is largely unknown.

The problem of nonpoint source pollution in agricultural catchments

The primary role of freshwater ecosystems in the biosphere is as conduits of water and nutrients from the continents to the sea (Bailey et al. 2004). Nevertheless, fluvial systems not only act like water receptors, but they have the capacity to transforms, retain and remove water nutrients affecting to its quality (Peterson et al. 2001; Alexander et al. 2009). Besides, they also support unique and complex ecological communities and often define the structure and functioning of the surrounding terrestrial ecosystems (Bailey et al. 2004). Because of this, and due to its critical role as human resources, during the last decades have had a high interest to develop management strategies that reduce the human activity impacts on them (e.g., NICOLAS European project (Nitrogen Control by Landscape Structures in agricultural environments - 1998); Framework Water Directive (2000/60/EC); CWSRF program (Clean Water State Revolving Fund- USEPA- 2009)).

Nowadays, intensive agriculture is globally recognized as a major nonpoint source pollution, contributing significantly to increase nitrogen (N) and phosphorus (P) concentrations in freshwater ecosystems (Mason et al. 1990; Carpenter et al. 1998a; Correll 1998; Baker and Richards 2003; Nord and Lanyon 2003; Conley et al. 2009; Kopacek et al. 2013). This pollution is more difficulty controlled than point source pollution and its reduction can only be achieve by appropriate management techniques, as for example, by the decrease of agricultural fertilizer inputs, the reduction of agricultural runoff and soil conservation practices.

The excessive N and P enrichment of freshwaters can result in serious eutrophication problems, with environmental consequences such as an increase in the abundance of algae and aquatic plants, hypoxia, fish kills and loss of biodiversity (Vitousek et al. 1997; Smith 2003) and consequences for human health (Townsend et al. 2003). In addition, the eutrophication can also have negative economic effects, so it can result in a loss of the services that aquatic ecosystems provide, such as the water resources for drinking, industry and agriculture, or the recreation use (Carpenter et al. 1998b).

In the Mediterranean region, and especially in the most arid zones such as the Iberian southeast, water has been one of the limiting factors of economic activity (Prat and Munné 2000). However, water transfer between catchments has allowed that the irrigated crops take up large areas and put a strong pressure on freshwater ecosystems, among other environmental problems (Martínez-Fernández et al. 2000; Esteve 2003; Martínez-Fernández and Esteve 2006). In fact, the irrigation surpluses and the leached of nutrients and other pollutants from crops generate serious problems of water quality (Gómez et al. 2005).

This problem is specially accused in the arid and semiarid regions, where the majority of aquatic ecosystems have low discharges that, among other effects, diminish their solute dilution capacity and, this make them especially sensitive to eutrophication (e.g., Álvarez-Cobelas et al. 2005; Arce et al. 2013). In this context, the interception and attenuation of nonpoint source pollution is an indispensable requirement to prevent and manage the eutrophication of aquatic ecosystems in general, and of agricultural catchments of the Iberian southeast in particular.

Wetlands

Wetlands are a common feature of many landscapes worldwide. Their study began in the 40s (Lindeman 1942; Odum, 1957) and although many definitions of the "wetland" term have been had since then (e.g., Cowardin et al. 1979; Howard-Williams 1985; Ramsar-Bureau 1988), in all of them the water is always the dominant factor that determines the presence of wetlands.

In general terms, wetlands are areas where water table level is at or near the surface and it is the factor determining the soil development and the types of plant and animal communities living in (Cowardin et al. 1979). They share properties of both terrestrial and aquatic systems, and of both lotic and lentic systems, being important ecotone zones, with a high biodiversity, that have also an important role such as metabolic regulators of both systems (Howard-Williams 1985; Wetzel 1990). Wetlands are waterfowl habitats that is, areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine waters, the depth of which at low tide does not exceed six metres (Ramsar-Bureau 1988). In 1988, González-Bernáldez adjusted the wetland term for its utilisation in arid and semiarid zones, and defined it as “formations that, not being rivers or lakes, represent a temporal or spatial positive water anomaly in the landscape”.

In arid and semiarid zones, scarce precipitations (with alternation of heavy rain events of short length and long drought periods), the high evapotranspiration (greater than precipitation during most months) and the infrequent runoff events and recharge of groundwater (Gasith and Resh 1999), determine that wetlands beeing singular systems in the landscape (González-Bernáldez 1989; Hollis 1990).

They are zones usually fed by groundwater flows from higher parts of the catchment (Hollis 1990) which, from a functional point of view, regulate the microclimate generating more humid and cooler environments (González-Bernáldez and Pérez 1988), and play a key role in the retention of sediments and contaminants, as well as, in the dynamics of nutrients at catchment scale (Gómez 1995).

Although these wetlands usually occupy small surfaces of land, they are systems with a high biological productivity (Hollis 1990), because they remain active in the summer, when the rest of the territory suffers an important water deficit. In this sense, wetlands are biogeographic anomalies, since they are the refuge for many vegetal and animal species (González-Bernáldez et al. 1987; González-Bernáldez et al. 1989).

In general, climate and geology determine wetland phenology and functioning (Alonso et al. 1985). In the Iberian southeast, the scarce precipitation and high evaporation characteristic of semiarid Mediterranean climate, together with the dominance of sedimentary marls easily eroded and salt rich determine the existence of wetlands that present a temporary hydrologic regime and a high salinity, which also determines the presence of halophytic plants. These wetlands are located in coastal zones (littoral marshes), in the most depressed part of large alluvial plains or associated to drainage systems (Ramirez-Díaz 1992; Gómez 1995; Ballester 2003a; Ballester 2003b).

Wetlands associated to drainage systems, in accordance with its location in the landscape and its hydrology, are designated with the anglosaxon term "slope wetlands" (Stein et al. 2004; Acreman 2005) (Fig. 1). In the Iberian southeast, these wetlands are located in the riverbed of small temporary streams "ramblas" that discharging in water courses of greater order (rivers or other "ramblas"). They locate specifically in zones where the topographic conditions allow subsurface water, from upper parts of the catchment, intersect the soil surface creating a zone of perennial or near-perennial moisture (Fig. 1). This subsurface water input is the main source of water and nutrients in these wetlands (Gómez 1995; Gómez et al. 2001), and it takes place in their upper part where helophytes, mainly Phragmites Australis, are abundant. Besides, they receive sporadic water inputs, as pulses, if precipitations or avenues take place.

The water flows downslope through the wetlands like a shallow sheet among halophytic shrubs, such as *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa* and *Suaeda vera*, which replace the reed *(Pragmites australis)* as the water salinity increases (Fig. 1). In addition, small patches of *Tamarix canariensis*, *Tamarix boveana* and *Juncus maritimus* often appear disperses. Between emergent vegetation, attached to sediments, is common the presence of biofilm (a complex community of bacteria, algae, fungi and protozoa) and two species of macrophytes, *Vaucheria dichotoma* and *Ruppia maritima* (Gómez 1995). Sometimes, subsurface water that creates these wetlands does not emerge in surface and thus, these wetlands only have subsurface flow. Nevertheless, the helophytic and halophytic vegetation is present.

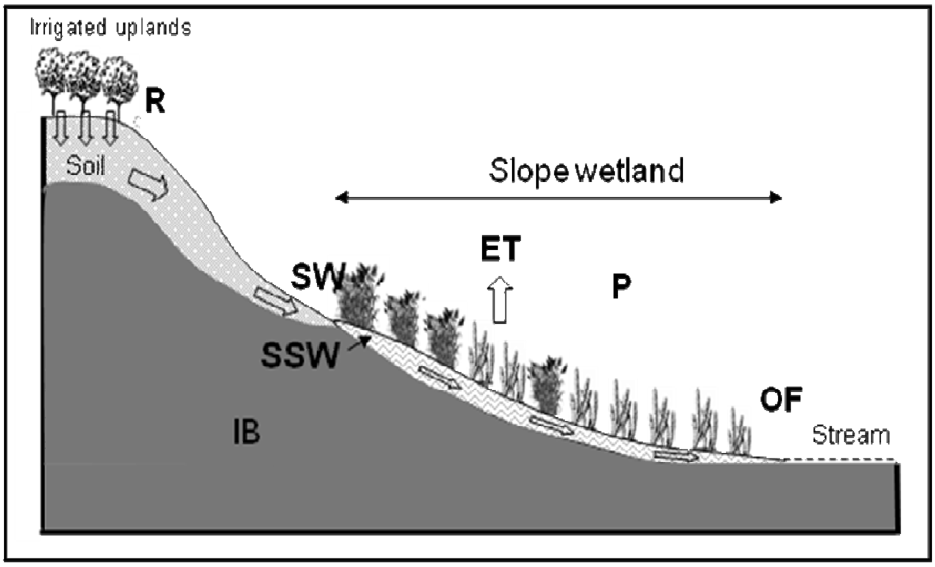

Fig.1. Vertical section view of a slope wetland showing the ways in which water can move into them or from them: R runoff water, SW surface water, SSW subsurface water, IB impermeable bed, ET evapotranspiration, P precipitation, OF surface and subsurface outflow to the river.

Regarding the temporal dynamics, these systems are influenced by droughts and avenues as natural perturbations (Gómez 1995; Gómez et al. 1995). In summer, surface water can disappear with the decrease of the water table level and the increase of the evaporation/evapotranspiration rate. In autumn, if there are strong precipitations, can occur avenues that supply water, nutrients, organic matter and sediments to the system, as well as, mobilise the channel materials.

Finally, it is necessary to emphasize that although these wetlands are singular elements in the Mediterranean landscape and especially, in the European context, as they have until 4 types of habitats of community interest (Mediterranean salt meadows (Juncetalia maritim); Mediterranean and thermo-Atlantic halophilous scrubs (Sarcocornetea fruticosi); Halo-nitrophilous scrubs (Pegano-Salsoletea) and

Mediterranean salt steppes (Limonietalia)), are subjected to a strong human pressure (Vidal-Abarca et al. 2000; Gómez et al. 2005). Its location, in sedimentary catchments with a high percentage of agricultural land use, its small size and the non-existent social recognition like singular ecosystems favour that suffer multitude of impacts with total impunity, such as the poured of rubbish and debris, traffic and the desiccation and/or roturación for its put in crop (Gómez et al. 2005).

The role of wetlands in controlling nonpoint source pollution

Wetlands are not only important ecosystems by their intrinsic values, but besides by the ecosystemic services that provide. Between these, it is necessary to mention the importance of the biogeochemical processes that take place in them and that determine their important function like natural filters of nutrients and other contaminants (e.g., Mitsch And Gosselink 2000; Fisher and Acreman 2004; Verhoeven et al. 2006).

In general terms, it is globally recognised that natural wetlands, in which riparian forests are included, and constructed wetlands reduce the flow of nutrients from terrestrial to aquatic ecosystems, being both used in the diffuse pollution treatment in agricultural, urban and even mining zones (Lowrance et al. 1984; Mitsch 1992; Mitsch et al. 2000; Braskerud 2002a; Braskerud 2002b; Álvarez-Rogel et al. 2006; Knox et al. 2008; Vymazal and Kröpfelová 2008; Ardón et al. 2010). In this context, the Water Framework Directive (2000/60/EC) emphasizes the role of natural wetlands as significant elements of the hydrological networks required to obtain the good ecological status for surface and subsurface waters. Nevertheless, we do not have to consider the natural wetlands as simple “waste water treatment plants”.

The main biological processes responsible of nutrient removal in wetlands are the assimilation by vegetation and microorganisms, that is a temporary removal of N and P, and the denitrification, microbiological process that supposes a permanent removal of N for the system (e.g., Reddy and Patrick 1984; Groffman et al. 1992). Another process directly involved in the P concentration decrease is its retention in sediments, being ocasionally this the main mechanism of removal (e.g., González-Alcaraz et al. 2012). In general, the effectiveness of all these processes depends on the vegetation type, the soil physicochemical conditions, the water temperature, the organic carbon and N-NO_3^- availability, the water table level or the current discharge (Howard-Williams 1985; Bowden 1987; Reddy et al. 1995; Pinay et al. 2007).

In the Iberian southeast, climatological conditions confer to wetlands a great potential to be considered effective elements in controlling the nonpoint source pollution. The high solar radiation and the soft temperature along the year facilitate that the biological processes involved in the nutrient transformation and elimination can remain active during more time, in comparison with wetlands of temperate zones (e.g., Spieles and Mitsch 2000; Pinay et al. 2007). On the other hand, its strategic location in the agricultural landscapes allows them intercept the surface and subsurface water flows, and act on the nutrient load, before they reach other aquatic systems. For example, previous studies realised in coastal marshes (Álvarez-Rogel et al. 2006; González-Alcaraz et al. 2012) have showed the efficiency of these humedales reducing the N and Pconcentrations. However, it is unknow the role that inland wetlands associated to drainage networks can have in controlling the diffuse pollution, given its lower size and the specific conditions that characterise them.

The study of this capacity not only allows to analyse the utility of these wetlands as tools for controlling diffuse pollution, improving the water quality of the aquatic ecosystems, but it puts in value the conservation of these systems. In fact, the conservation and/or restoration of inland wetlands in landscapes essentially agricultural, where different human activities compete by the land, only is possible by putting them in value and with a social recognition of the services that these offer.

Objectives and structure of the present memory

The present memory pretends to provide information on distinct aspects related with the capacity of wetlands associated to drainage systems of the Iberian southeast to remove N and P of water. Such information will be useful to know the role that they have in controlling nonpoint source pollution in agricultural catchments and thus, in the improvement of water quality, what will suppose its recognition and revalorization like natural system. In this context four general objectives are established:

- To know the N and P retention efficiency of wetlands associated to drainage systems, as well as, its temporal variability over a hydrological cycle.
- To know if exist differences in the N and P retention efficiency between the surface and subsurface compartments of these wetlands.
- Analyse the environmental factors that affect to N and P retention in these systems.
- Analyse the spatial variability, along the longitudinal axis of wetlands, of the N and P retention efficiency in both compartments.

Basing on the mentioned objectives, the present memory is structured in three chapters that have written as independent publications and whose specific objectives are:

Chapter 1

The objectives of this chapter are 1) to quantify the retention efficiency of the different N forms (nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), total organic nitrogen

(TON-N) and total nitrogen-N (TN-N)) in the surface component of two Mediterranean wetlands affected by agricultural runoff, 2) to analyse the temporal variability of the retention efficiency for the different N forms over a hydrological cycle, and 3) to examine the effect of the hydrology and the N load on retention efficiency.

The content of this chapter has been published in the following article:

García-García V, Gómez R, Vidal-Cover MR, Suárez ML (2009) Nitrogen retention in natural Mediterranean wetland-streams affected by agricultural runoff. Hydrology and Earth System Sciences 13: 2359-2371

Chapter 2

The objectives of this chapter are 1) to quantify the retention efficiency, during a hydrological cycle, of the different N forms (NO_3^--N, NH_4^+-N, TON-N and TN-N) in the subsurface component of two Mediterranean wetlands affected by agricultural runoff, 2) to examine the effect of the hydrology and the N load on the retention efficiency, and 3) to compare the N retention between the surface and subsurface components, and to establish if there are differences.

The content of this chapter has been published in the following article:

García-García V, Gómez R, Vidal-Cover MR, Suárez ML (2013) Subsurface N retention in two Mediterranean wetland-streams affected by agricultural runoff. Wetlands 33: 597-608

Chapter 3

The objective of this chapter is 1) to examine the spatial variability, along the longitudinal axis of wetlands, of retention efficiency for different N (NO_3^--N, NH_4^+-N, TON-N and TN-N) and P (soluble reactive phosphorus (SRP), phosphorus particulado (P-Part) and total phosphorus (PT)) forms in the surface and subsurface components of two Mediterranean wetlands affected by agricultural runoff.

This chapter corresponds with the article submitted for his review and publication:

García-García V, Gómez R (submitted) Spatial variability in N and P retention through reaches in two Mediterranean slope wetlands. Ecological engineering.

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Chapter 1

Nitrogen retention in natural Mediterranean wetland-streams affected by agricultural runoff

V García-García, R Gómez, MR Vidal-Abarca and ML Suárez

Department of Ecology and Hydrology, Faculty of Biology, University of Murcia, Campus of Espinardo, 30100 Murcia, Spain

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Abstract

Nitrogen retention efficiency in natural Mediterranean wetland-streams affected by agricultural runoff was quantified and the effect of the temporal variability and hydrological/chemical loading was examined from March 2007 to June 2008 in two wetland-streams located in Southeast Spain. Nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), total nitrogen-N (TN-N), total organic nitrogen-N (TON-N) and chloride (Cl^-) concentrations were analyzed to calculate nitrogen retention efficiencies. These wetland-streams consistently reduced water nitrogen concentration throughout the year with higher values for NO_3^--N (72.3%), even though the mean value of inflow NO_3^--N concentrations was above 20 mg l^{-1}. Additionally, they usually acted as sinks for TON-N (8.4%), but as sources for NH_4^+-N. Over the entire study period, the Taray and Parra wetland-streams were capable of removing on average 1.6 and 0.8 kg NO_3^--N a day^{-1}, respectively. Retention efficiencies were not affected by temperature variation. NO_3^--N retention efficiency followed a seasonal pattern with the highest retention values in summer (June-September). The temporal variability for NO_3^--N retention efficiency was positively and negatively explained by the hydrologic retention and the inflow NO_3^--N concentration ($R^2_{adj}=0.815$, $p<0.01$), respectively. No significant regression model was found for TON-N and NH_4^+-N. Finally, the conservation of these Mediterranean wetland-streams may help to not only improve the surface water quality in agricultural catchments, but to also achieve good ecological status for surface waters, this being the Water Framework Directive's ultimate purpose.

1 Introduction

Nitrogen is an essential nutrient for aquatic ecosystem functioning. Its variation influences community structure, microbial activity and primary production (Pringle 1990; Peterson et al. 2001; Dodds et al. 2002). In recent years however, nitrogen (N) concentrations have increased in many areas as a result of human activities and have important negative effects on natural ecosystems (Townsend et al. 2003; Niyogi et al. 2004). Therefore, a great deal of attention has been paid to the movement (fluxes) and transformation of N, especially in streams (Peterson et al. 2001; Kemp and Dodds 2002; Gücker and Boëchat 2004).

Agricultural runoff is an important source of non point pollution of aquatic ecosystems, causing eutrophication through nutrient load enrichment (Peterjohn and Correll 1984; Mitsch et al. 2005). Unlike point source pollution, diffuse pollution is less easily controlled and its reduction can only be achieved by appropriate land management techniques.

Over the last few decades, much interest has been manifested in specific natural systems, such as riparian zones which are able to reduce or buffer the flux of N from terrestrial to aquatic ecosystems (Lowrance et al. 1984; Groffman et al. 1992; Sabater et al. 2003). In general, wetlands can improve water quality through physical, chemical and biological processes that remove N from water (Howard-Williams 1985). This is possible because they have zones of high primary productivity in surface environments and decomposition in sediments that create coupled aerobic and anaerobic transformations of N molecules that pass through them (Bowden 1987). The role of wetlands in removing N from runoff surface waters is globally recognized (Lowrance et al. 1984; Fisher and Acreman 2004), but the extreme variability of biological and hydrological processes make it difficult to predict the efficiency of N retention of the different types of wetlands.

Nitrogen retention efficiency in constructed wetlands has been extensively studied for wetlands to be used in conjunction with agricultural drainage and wastewater treatment systems (Spieles and Mitsch 2000). However, few studies have analyzed nutrient retention efficiencies in natural wetlands (Jordan et al. 2003; Vellidis et al. 2003; Fisher and Acreman 2004; Knox et al. 2008), despite some studies demonstrating their utility in water quality control on the catchment scale (Mitsch 1992; Mitsch et al. 2005; Chavan et al. 2008). Indeed, the European Framework Directive (2000/60/EC) emphasizes the role of wetlands as significant elements of the hydrological networks required to obtain a “good water status” for surface and ground waters (Wetlands Horizontal Guidance 2003).

In the Southeast Iberian Peninsula (Spain), the presence of small wetland-streams is a typical feature of the Mediterranean landscape of sedimentary catchments (Gómez et al. 2005). These wetlands, which are associated with stream drainage systems, intercept the runoff waters originating from the agricultural catchments in which they are located. This spatial arrangement converts wetland-streams into natural tools to control non point pollution. These agricultural areas are typically fertilized with N inorganic salts (KNO_3) with a load of 250-300 kg N ha^{-1} $year^{-1}$.

Apart from the studies on N retention in Mediterranean streams (Martí and Sabater 1996; Sabater et al. 2000; Martí et al. 2004; Von Schiller et al. 2008), there are virtually no studies related with Mediterranean wetlands.

Unlike temperate wetlands, a feature of Mediterranean wetland-streams and other arid and semi-arid aquatic systems is the hydrological intermittency (Gasith and Resh 1999; Acuña et al. 2005) which strongly influences the structure and functioning of aquatic ecosystems, including N dynamic (Bernal et al. 2005; Von Schiller et al. 2008; Gómez et al. 2009). Moreover, the N concentration and water discharge in aquatic systems affected by agricultural runoff inputs likely show wide temporal fluctuations, mainly due to crop irrigation practices. Over longer time scales, the nature and extent of N input into wetlands will likely affect attenuation processes. By way of example, riparian zones that have been subject to long-term nitrate inputs may have attenuation capacities that differ from non nitrate enriched areas (Groffman et al. 1992). Many studies reported a negative effect of high N concentrations and discharges on N retention efficiency in wetlands (Emmett et al. 1994; Spieles and Mitsch 2000; Knox et al. 2008). In fact, high N concentrations may have a saturation effect on N microbial and plant uptake (Sabater et al. 2003; Bernot and Dodds 2005). On the other hand, high water discharges provide short retention times and low surface areas for N exchange per unit volume of water (Peterson et al. 2001; Pinay et al. 2002).

The two objectives of this study were to quantify the N (NO_3^--N, NH_4^+-N, TN-N and TON-N) retention efficiency in Mediterranean wetland-streams affected by agricultural runoff and to examine the effect of the temporal variability and hydrological/chemical loading on N retention.

An understanding of the N retention capacity of the Mediterranean wetland-streams receiving agricultural runoff is important for several reasons: it may help to determine the key factors driving N retention in these systems; it allows better predictions of how N retention in wetland-streams will vary in response to fluctuations of hydrologic/chemical loading and it allows researchers and managers to design better management plans to control non point pollution in agricultural catchments.

2 Materials and methods

2.1 Study site

The study was carried out in two natural wetland-streams, the Taray and Parra wetlands, located in the Murcia Region in Southeast Spain (Fig. 1). The climate of the study area is semiarid Mediterranean with temperate winters and hot, dry summers. Average annual precipitation is 300 mm and the average annual temperature is close to 18ºC.

Wetland-streams are situated at the outlet of small catchments (the mean altitudes are 207 and 172 m over sea level for the Taray and Parra wetlands, respectively) and collect runoff waters from agricultural lands and natural surrounding areas (Fig. 1, Table 1). Surface water flows through the Taray and Parra wetlands and finally, water leaves them via an intermittent channel that flows into the Salada and Parra streams, respectively (Fig. 1). Both wetland-streams are intermittent with periods of low discharge (usually during summer) or even drought periods. Geomorphological features and discharge data are shown in Tables 1 and 2, respectively.

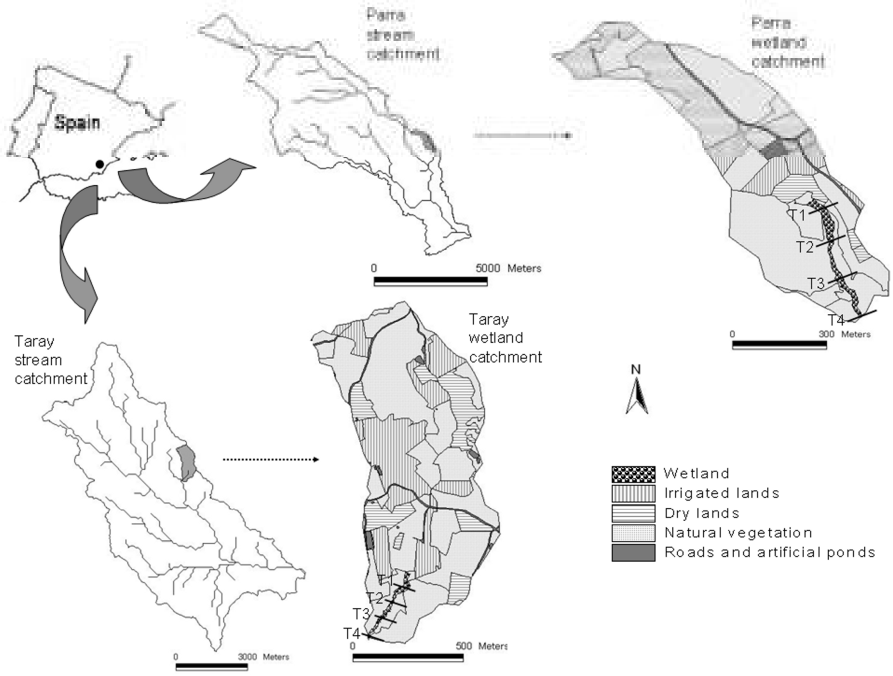

Fig. 1. Location of the studied wetland-streams and their catchments. T1, T2, T3 and T4 represent the four transects on each wetland where samples were collected.

The wetland-streams' catchments are characterized by impermeable sedimentary marls (from the Miocene) with a considerable gypsum content (calcium sulfate) and halite (sodium chloride). As a result of this lithology, water conductivity is very high (Table 2) and wetland-stream sediments have a considerable clay and silt content. Natural vegetation in catchments is scarce and dominated by Mediterranean shrubs, including species like *Stippa tenacissima*, *Lygeum spartum* and *Thymus hyemalis*. Wetland-stream plant communities are composed of helophitic species like *Phragmites australis* and *Juncus maritimus,* and halophytic species like *Suaeda vera*, *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa*, in the lower flooded areas.

P. australis is located in the upper-part of the wetlands with a plant cover that ranges from 47.2% to 58.7% for the Taray and Parra wetlands, respectively. *J. maritimus* only appears in small patches in the lower part of the Taray wetland. With the exception of small patches of *Vaucheria dichotoma,* aquatic macrophytes are absent. Periphyton communities are frequent on fine substrates.

Table 1. Surface area, land uses at wetland-stream catchments and geomorphological features recorded in the wetland-streams during the study period. [a] Calculated from GIS data. [b] Irrigated lands included fruit trees and vegetables (with irrigation and fertilizer inputs). [c] Dry lands included almond and olive trees (without irrigation and fertilizer inputs).

	Taray	Parra
Wetland-stream catchment		
Total area (ha) [a]	74.5	33.2
Irrigated lands (%) [a, b]	24.1	10.8
Dry lands (%) [a, c]	13	24.6
Natural vegetation (%) [a]	60.5	61.8
Roads and artificial ponds (%) [a]	2.4	2.8
Wetland-stream		
Total area (ha) [a]	0.5	0.7
Surface flow length (m)	300	300
Surface flow width (m)	3.4-7.1	2.3-13.4
Surface flow depth (cm)	0.5-10	0.5-10

2.2 Methods

To determine the wetland-stream retention efficiencies for NO_3^--N, NH_4^+-N, TN-N and TON-N, four sampling transects were located on each wetland, perpendicularly to the water flow direction and with a separation of approximately 100 m (Fig. 2). Sampling transects were opened through vegetation areas to reach the surface water. Surface water samples were collected once a month from the different transects, from March 2007 to March 2008 (13 sampling dates) in the Taray wetland and from April 2007 to June 2008 (15 sampling dates) in the Parra wetland (Fig. 2). The four transects of Parra wetland were dry from July to September 2007, while in the Taray wetland surface water only disappeared in the transect 3 during August and September 2007 (Fig. 2).

Surface water samples were collected with plastic syringes (100 ml) as the water was so shallow (Table 1), and were stored in previously acid-washed polyethylene bottles (500 ml) under dark and cold conditions until they were analyzed at the laboratory. The number of samples per transect varied between 1 and 4, depending on the water sheet width (Fig. 2). The total number of samples per sampling date ranged from 7 to 11 and from 10 to 13 for the Taray and Parra wetlands, respectively. Air and water temperatures, salinity and conductivity (conductivity meter Tetracon 325; WTW, Munich, Germany) and the presence of macrophytes species or periphyton communities were also recorded at each transect.

The outlet discharge was estimated for both wetland-streams as the product of the average water velocity (current meter MiniAir2; Schiltknecht Co, Zürich, Switzerland) and the cross-sectional area at the wetland outlets (Transect 4, Fig. 2). It was not possible to measure the inlet discharge because of the diffuse surface water inputs to the wetlands.

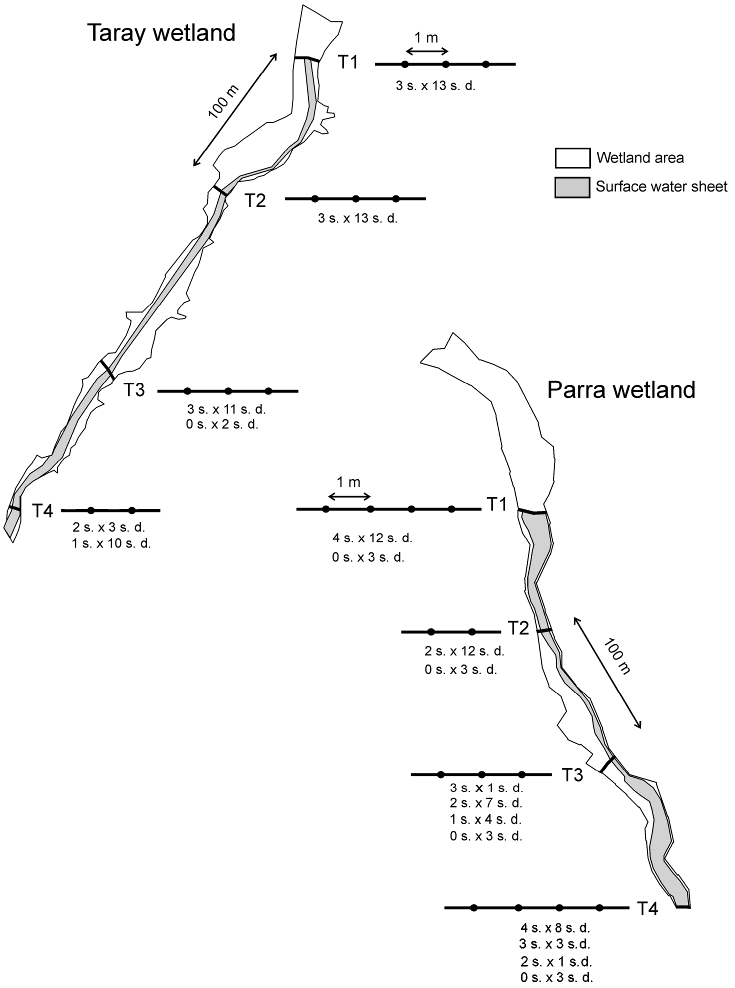

Fig. 2. Location of the four sampled transects in the studied wetland-streams. In each transect (black lines) the number of samples (s.) collected in the different sampling dates (s. d.) are shown. 0 samples mean that the transect was dry.

The precipitation data were obtained from the two nearest thermo-pluviometric stations to the studied wetlands (SIAM; Servicio de Información Agrometeorológica, Región de Murcia), Fortuna station that was located approximately 0.4 km from Taray wetland and Abanilla station that was located approximately 5.5 km from Parra wetland.

2.3 Chemical analyses

Water samples were analyzed for N dissolved forms within 24 hours of collection. They were filtered through glass-fiber filters (Whatman GF/C, 1.2 μm nominal pore size; Whatman International Ltd., Maidstone, England). NO_3^--N concentration was measured by a colorimetric method following cadmium reduction to nitrite-N (NO_2^--N) (Wood et al. 1967). NO_2^--N concentration was analyzed by diazotization (Strickland and Parsons 1972). NO_3^--N concentration was estimated by subtracting the NO_2^--N concentration obtained by diazotization. NH_4^+-N concentration was measured by the phenyl-hypochlorite colorimetric method (Solorzano 1969). Dissolved inorganic nitrogen (DIN) was calculated as the sum of the NO_3^--N, NO_2^--N and NH_4^+-N concentrations.

Total nitrogen concentration (TN-N) was measured on unfiltered and frozen samples. These samples were digested to NO_3^--N using potassium persulfate (D'Elia 1977) and were analyzed by cadmium reduction using an automated ion analyzer (EasyChem Plus, Systea Analytical Technologies, Italy). TON-N concentration was estimated by subtracting the DIN concentration from the TN-N concentration. Chloride concentration (Cl^-) was analyzed within 48 hours of collection by the silver nitrate volumetric method (APHA 1985).

2.4 Retention calculations

Chloride was used to calculate N retention in the wetland-streams (e.g., Simmons et al. 1992; Sabater et al. 2003). As a passive tracer, Cl^- undergoes dispersion, dilution and diffusion, but is not significantly removed from solutions and consequently, its movements largely track water flow. Thus, the variations in Cl^- concentration allow the detection of possible dilution (by lateral or subsurface water inputs) or solute concentration (by evapotranspiration) that also affects N forms.

An input-output nutrient budget for a wetland depends on a hydrological budget which in simple terms we assumed for the studied wetland-streams as $SW_{in} = E + SW_{out}$, where SW_{in} is the inflow surface water, E is the evapotranspiration and SW_{out} is the outflow surface water. With the exception of evapotranspiration as a water output, we assumed no hydrological inputs and outputs through the studied wetland-streams. Piezometric levels and subsurface Cl^- concentration data (not showed in this paper) together with the surface water Cl^- concentrations suggested that groundwater inputs (shallow subsurface flow sources) and outputs (surface water infiltration) through the stream-wetlands were negligible. Only in two occasions (August and September 2007, in the Taray wetland), surface water was infiltrated (T3) but few meters downwetland it went back into the wetland surface (before T4).

Thus, retention efficiency (%R) was calculated for the different N forms (NO_3^--N, NH_4^+-N, TN-N and TON-N) on each sampling date by considering, Eq. 1 (Trudell et al. 1986):

$$\%R = (1- (N/Cl^-_{out} / N/Cl^-_{in})) \times 100 \tag{1}$$

N/Cl^-_{in} and N/Cl^-_{out} are the concentration ratios of both solutes in the inlet (T1) and outlet (T4) of both wetland-streams, respectively. Although to estimate N retention were only used N and Cl^- data registered in T1 and T4, data from the rest of sampling transects (T2 and T3) were used to check any possible water input and to control the applicability of the used equations (described above and below).

%R is the percentage of the N removed by the wetlands in relation to the inflow of N. A positive retention value indicates that the inflow N/Cl^- ratio was higher than the outflow N/Cl^- ratio. Under this circumstance wetland-streams were N sinks. On the contrary, a negative retention value indicates that the outflow N/Cl^- ratio was higher than the inflow N/Cl^- ratio and wetland-streams were N sources. The outflow N load (mg N day^{-1}) was calculated as the product of outflow N concentration (mg l^{-1}) by outlet discharge (l s^{-1}). Under the previously described assumption, the percentage of retention (%R) was applied to the outflow N load to estimate the inflow N load (mg N day^{-1}). The N net removal was calculated as follows:

$$\text{Nitrogen net removal} = \text{inflow N load} - \text{outflow N load} \quad (2)$$

Finally, the net hydrologic retention for each sampling date in both wetland-streams was calculated by considering, Eq. 3, used by Stanley and Ward (1997):

$$\text{Net hydrologic retention} = (\text{inlet discharge} - \text{outlet discharge}) / \text{inlet discharge} \quad (3)$$

The net hydrologic retention was estimated as an indirect measurement of the water residence time inside the wetland. Positive values of the net hydrologic retention (< 1) indicate that the discharge diminishes as surface water flows through the wetland

and as a consequence, the water velocity diminishes and the water residence time increases. The net hydrologic retention is 1 when the wetland is dry. If the discharge does not change through the wetland, the net hydrologic retention is 0. A negative value indicates that the outlet discharge is higher than the inlet discharge as result of surface or subsurface water inputs. Because it was not possible to measure the inlet discharge, due to the diffuse water inputs to the wetlands, this was calculated for each sampling date as follows:

$$\text{Inlet discharge } (\text{l s}^{-1}) = \text{outlet discharge} * \text{Cl}^-_{out} / \text{Cl}^-_{in}. \qquad (4)$$

2.5 Statistical analyses

The coefficient of variation (CV) for inflow N concentrations and retention efficiencies was used as an indicator of their temporal variability throughout study period. The relationship between N retention efficiency and the physical, chemical and hydrological parameters was evaluated using Spearman correlations with the SPSS software rel.15.0.1 for Windows (SPSS Incorporated, Chicago). Seasonal differences in N retention were analyzed using analysis of variance (one-way ANOVA), followed by Tukey's post-hoc test with the SPSS software. Months were grouped as follows: spring (March-May), summer (June-September), autumn (October-November) and winter (December-February). Multiple linear regression analyses were used to calculate the best fitting regression model that explains the N retention in the studied wetland-streams. The percentages of NO_3^--N retention were transformed prior to regression analysis with the arcsin transformation $y = \arcsin(\sqrt{p})$, where p is the percentage expressed as a proportion, in the range 0-1. The transformed variable (y) was fitted to the multiple regression analysis. Arcsin transformation is usually applied to binomial data in order to approximate normal variance. However, our purpose here was to bind the limits of prediction by multiple regression into the bounds 0-1 (0-100%) as the result of transforming back to original units is constrained to the range 0-1. Not transforming the variable may result on the unrealistic case of multiple regression predicting >100% retention, in some cases. The multiple linear regression analyses were performed with R rel.2.6.0 for Windows (R Development Core Team, Vienna, Austria).

3 Results

3.1 Inflow water characterization

Figure 3 shows the variation of accumulated total precipitation between consecutive sampling dates and the outlet discharge in both wetland-streams during the study period. Temporal variability of the accumulated total precipitation was high and the maximum values were registered mainly in months of spring and fall (March, April and October). The outlet discharge also differed vastly between study months (CV=87.7% and 68.2% in the Taray and Parra wetlands, respectively) but their highest and lowest values did not always correspond with increases or decreases in the precipitation, respectively. Despite the high temporal variability of the outlet discharges, the mean values in both wetland-streams were similar (Table 2).

Table 2. Mean, median, 10th and 90th percentile values for solute concentrations, conductivity and temperature of inflow water to wetland-streams. The inlet/outlet discharges and the net hydrologic retention values are also shown. Inlet discharge values were estimated by Eq. 4 (method section). n=13 and n=12 in the Taray and Parra wetlands, respectively. * n=11.

	Taray wetland-stream				Parra wetland-stream			
	Mean	Median	P10	P90	Mean	Median	P10	P90
TN-N (mg l^{-1})	23.9	23.6	20.8	27.4	29.7	28.7	13.6	46.9
NO_3^--N (mg l^{-1})	21.5	21.4	18.4	24.4	27.4	27.1	12.7	42.8
TON-N (mg l^{-1})	2.4	2.1	0.5	4.7	2.3	0.7	0.1	7.8
NH_4^+-N (mg l^{-1})	0.01	0.01	0.003	0.03	0.01	0.01	0.001	0.03
Cl^- (g l^{-1})	3.2	3.3	2.9	3.5	3.5	3.5	2.5	4.3
Conductivity (mS cm^{-1})	17.3	17.7	15.5	18.5	15.6	15.1	13.2	18.4
Water temperature (ºC)	15.8	16.1	9.6	22	14.7	14.8	10.8	17.4
Inlet discharge (l s^{-1})	1 *	0.7 *	0.1 *	2.6 *	0.8	0.8	0.1	1.4
Outlet discharge (l s^{-1})	0.6 *	0.4 *	0.1 *	1.4 *	0.7	0.7	0.1	1.4
Net hydrologic retention	0.5 *	0.5 *	0.4 *	0.5 *	0.1	0.1	0.002	0.2

Table 2 compiles the physicochemical characterization of the inflow water in the wetland-streams during the study period. Although the mean value for inflow TN-N concentration was higher in the Parra than in the Taray wetland (Table 2), the relative contribution of N forms in the inflow water was similar in both wetlands (90.4%, 9.5%, 0.1% and 92.6%, 7.3%, 0.1% as NO_3^--N, TON-N and NH_4^+-N, respectively). The highest variability in the range of inflow N concentrations throughout the study period corresponded to the Parra wetland.

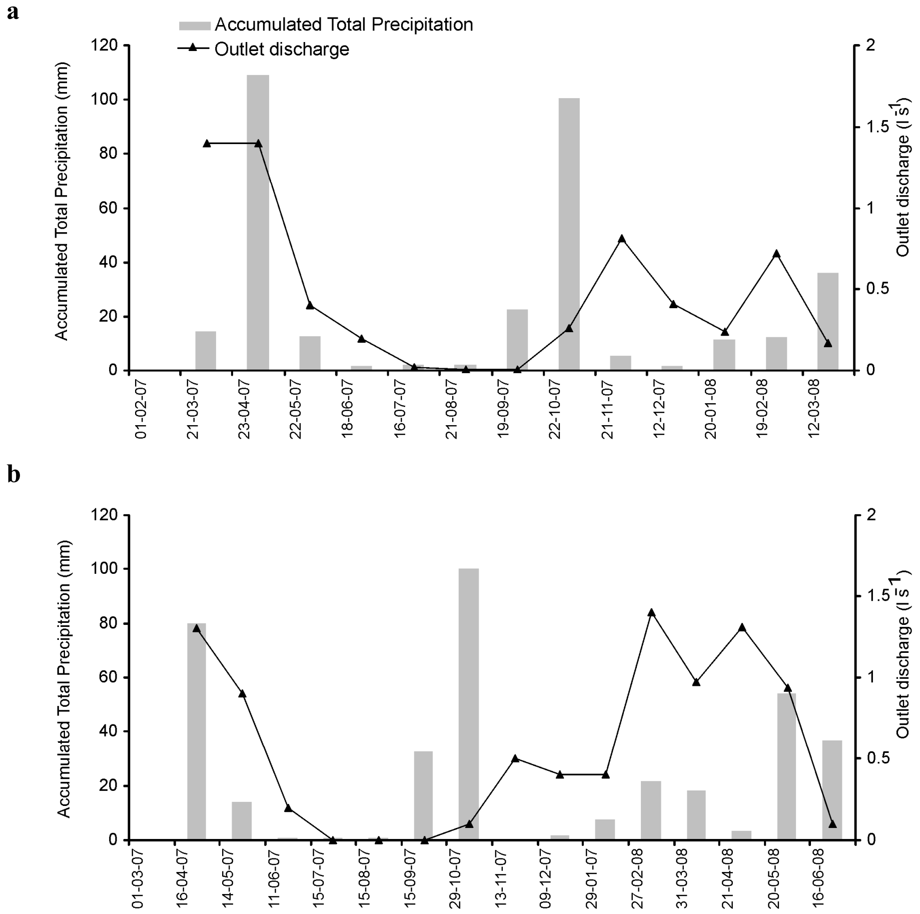

Fig. 3. Accumulated total precipitation (between consecutive sampling dates) and outlet discharge registered during the study period in the (**a**) Taray and (**b**) Parra wetland-streams.

The inflow NO_3^--N concentrations in the Taray wetland were consistently similar throughout the study period (CV=8.6%, n=13), while a higher temporal variability was noted for the Parra wetland (CV=37.1%, n=12) (Fig. 4). This difference between both wetland-streams was mainly influenced by the sharp increase of inflow NO_3^--N concentration (30-43 mg l^{-1}) registered from March to June 2008 in the Parra wetland (Fig. 4). The inflow TON-N and NH_4^+-N concentrations varied considerably among the study months (Fig. 4). The CV values for TON-N were 58.0% (n=13) and 131.3% (n=12) in the Taray and Parra wetlands respectively and were 83.6% (n=13) and 117.2% (n=12) for NH_4^+-N.

The mean value for inflow Cl^- concentration was high and similar in both wetland-streams (Table 2). As NO_3^--N, inflow Cl^- concentrations were similar throughout the study period in the Taray wetland (CV=6.7%, n=13), while they showed a greater temporal variability in the Parra wetland (CV=17.6, n=12) (Fig. 4). On the other hand, decreases of the inflow Cl^- concentration generally coincided with increases of the inflow NO_3^--N concentration in the Parra wetland (Fig. 4).

The mean value of the net hydrologic retention was higher in the Taray wetland than in the Parra wetland (Table 2), while their temporal variability was higher in the Parra wetland (CV=84%, n=12) than in the Taray wetland (CV=14%, n=11).

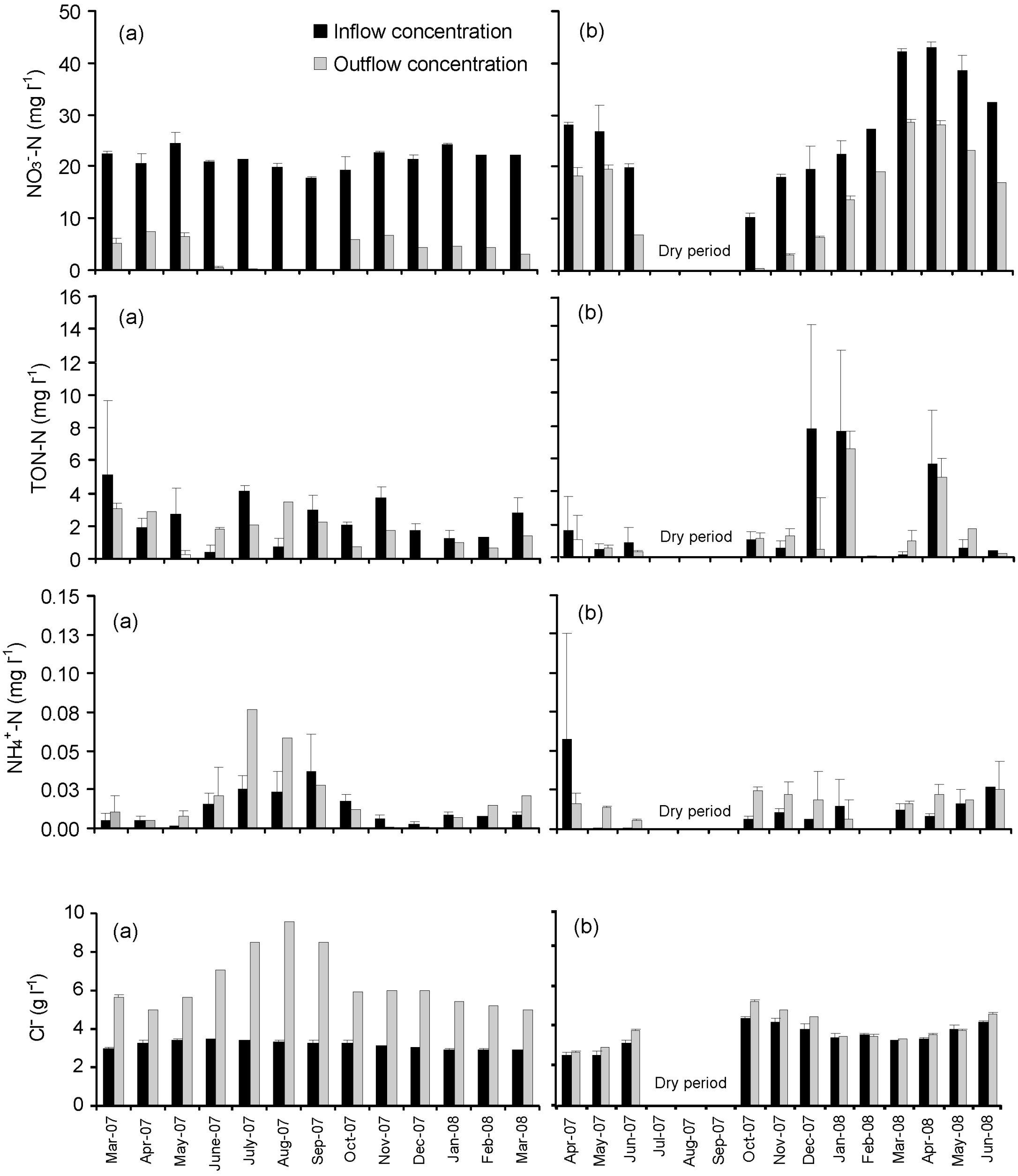

Fig. 4. Temporal variation of the inflow/outflow NO_3^--N, TON-N, NH_4^+-N and Cl^- mean concentrations (+SD) in the (**a**) Taray and (**b**) Parra wetland-streams, over the study period.

3.2 Nitrogen retention efficiencies

When all the sampling data from both wetland-streams were considered, the mean retention efficiency for TN-N was 70.1% (median value=82.9%, n=25) and it was higher in the Taray than in the Parra wetland (Table 3). Both wetland-streams showed the highest retention efficiency for NO_3^--N, followed by TON-N and NH_4^+-N (Table 3). The mean retention efficiency for NO_3^--N was 72.3% (median value=84%, n=25), ranging from 31.7% to 100%. However, the mean retention efficiency and net removal for NO_3^--N was consistently higher in the Taray wetland than in the Parra wetland (Table 3).

The retention efficiency for TON-N was low with a mean value of 8.4% (median value=56.1%, n=25) and ranged from -437% to 99.5%. The mean retention efficiency and the net removal were significantly higher in the Taray wetland than in Parra wetland (Table 3). There was not removal of TON-N from the water of both wetland-streams on 6 of the 25 sampling dates, as show the existence of negative retention values (Fig. 5). On these occasions, the TON-N/Cl^- ratio was higher at the outlet than at the inlet of both wetland-streams, denoting TON-N exportation.

Ammonium-N was not removed from water, but was exported instead on the majority of the sampling dates (13 of 25) (Fig. 5). The mean retention efficiency was -96.7% (median value=-3.2%, n=25), ranging from -1537.5% to 96.0%. As same as for TON-N, the mean retention efficiency was only positive in the Taray wetland and the net removal was also higher in this wetland (Table 3).

Table 3. Concentration, load, net removal, and retention efficiency for TN-N, NO_3^--N, TON-N, and NH_4^+-N registered at inflows and outflows of the wetland-streams. Values are the mean ± standard deviation based on the data collected over the study period (n=13 and n=12 in the Taray and Parra wetlands, respectively). * n=11.

	Concentration (mg l^{-1})		Load (mg m^{-2} d^{-1})			Retention efficiency (%)	Median retention efficiency (%)
	Inflow	Outflow	Inflow	Outflow	Net removal		
Taray wetland-stream							
TN-N	23.9 ± 2.4	5.4 ± 2.5	428 ± 383 *	72 ± 84 *	356 ± 309 *	87.9 ± 7	88.2
NO_3^--N	21.5 ± 1.9	3.8 ± 2.7	378 ± 321 *	54 ± 58 *	324 ± 269 *	90.4 ± 7.4	89.5
TON-N	2.4 ± 1.4	1.6 ± 1.1	49.3 ± 68.5 *	18 ± 27.5 *	31.3 ± 48.3 *	43.9 ± 69.7	71.2
NH_4^+-N	0.013 ± 0.01	0.02 ± 0.02	0.11 ± 0.08 *	0.08 ± 0.08 *	0.03 ± 0.06 *	11 ± 85.6	31
Parra wetland-stream							
TN-N	29.7 ± 10.4	17.1 ± 10	307.5 ± 251.9	186.8 ± 176.1	120.7 ± 81.5	50.9 ± 21.9	36.4
NO_3^--N	27.4 ± 10.2	15.4 ± 9.4	287.4 ± 237	171.6 ± 162	115.8 ± 79	52.8 ± 22.6	39.8
TON-N	2.3 ± 3	1.6 ± 2	19.8 ± 29.6	14.9 ± 22	4.9 ± 15.4	-30 ± 151	19.6
NH_4^+-N	0.013 ± 0.016	0.016 ± 0.008	0.14 ± 0.3	0.13 ± 0.11	0.01 ± 0.2	-213.4 ± 447.6	-49.04

3.3 Temporal variability of N retention efficiencies

The temporal variability of the retention efficiencies for NO_3^--N was higher in the Parra wetland than in the Taray wetland; CV=42.7% (n=12) and CV=8.2% (n=13), respectively (Fig. 5). Retention efficiencies for NO_3^--N increased during the summer (June-September) in both wetland-streams (Fig. 5). However, differences among seasons were only statistically significant in the Taray wetland (one-way ANOVA, F=29.9, $p<0.05$). The scarcity of data during summer in the Parra wetland (drought period) could be the reason of the absent of statistical significance for this wetland. The maximum NO_3^--N retention values (99.9% and 96.0%) were recorded in August and October in the Taray and Parra wetlands, respectively (Fig. 5).

The temporal variability of the retention efficiency for TON-N was higher than that for NO_3^--N (CV=158.8%, n=13 and CV=502.9%, n=12 in the Taray and Parra wetlands, respectively) and a seasonal pattern was not detected (Fig. 5). Retention efficiency ranged from –140% to 99.5% and from –437.4% to 95% in the Taray and Parra wetlands, respectively (Fig. 5).

As same as for TON-N, NH_4^+-N retention efficiencies varied considerably throughout the study period (CV=779.4%, n=13 and CV=209.7%, n=12 in the Taray and Parra wetlands, respectively) and no seasonal pattern was observed (Fig. 5). Negative NH_4^+-N retention values were recorded in many months, particularly in the Parra wetland (Fig. 5).

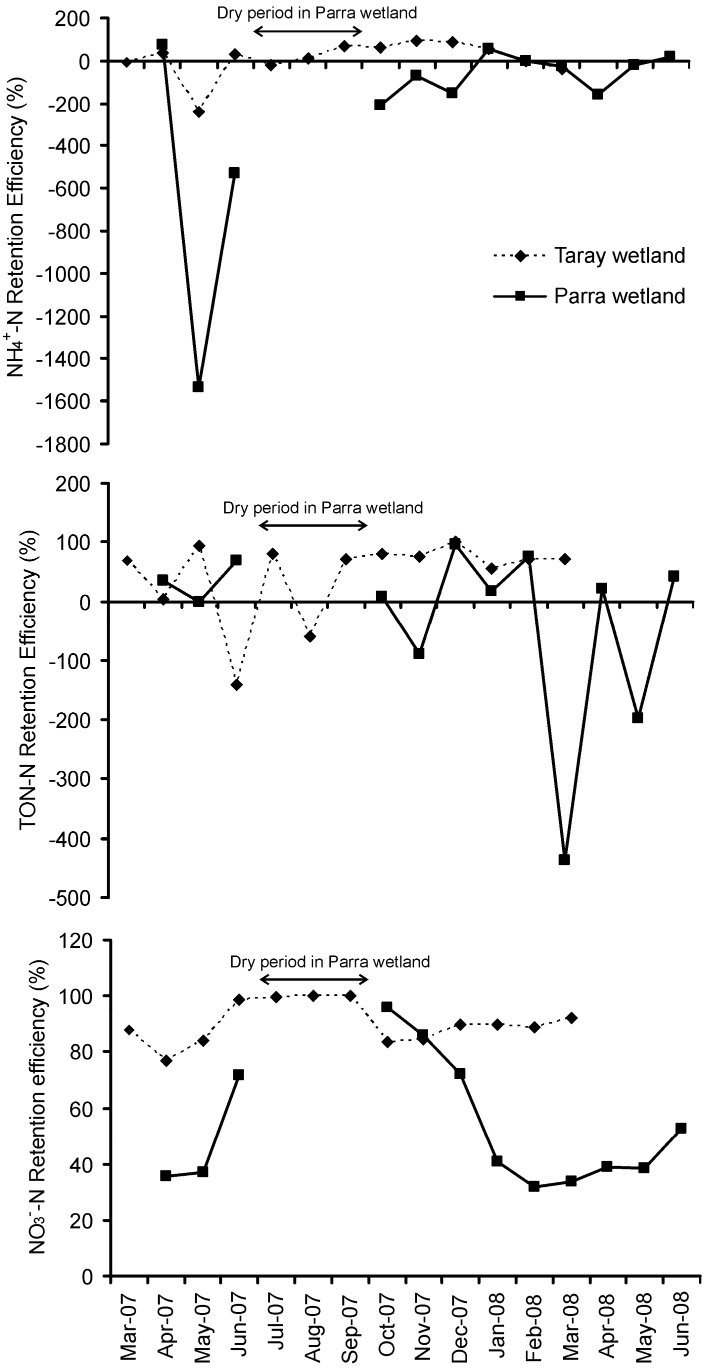

Fig. 5. Temporal variation of NH_4^+-N, TON-N and NO_3^--N retention efficiencies in the Taray and Parra wetland-streams.

3.4 Effect of environmental factors on N retention efficiencies

Table 4 shows the results of the Spearman correlations performed to evaluate the relationship between N retention efficiency and different environmental factors: inlet discharge, hydrologic retention, inflow N concentration, inflow load, and water and air temperatures.

The strongest relationship found was between NO_3^--N retention efficiency and net hydrologic retention, which was positive (Table 4). TON-N retention efficiency was also positively correlated with the net hydrologic retention (Table 4). In contrast, NH_4^+-N retention efficiency was not correlated with this variable (Table 4).

Nitrate-N retention efficiency was negatively correlated with the inflow NO_3^--N concentration and the inlet discharge, whereas TON-N and NH_4^+-N retention efficiencies were positively correlated with the inflow TON-N and NH_4^+-N concentrations, respectively (Table 4).

Finally, the multiple linear regression analysis showed that 81.5% of temporal variability for the NO_3^--N retention efficiency was explained by the net hydrologic retention and the inflow NO_3^--N concentration. This model was positive for the net hydrologic retention and negative for the inflow NO_3^--N concentration with a high level of significance (NO_3^--N retention efficiency = (sen (1.149 + 0.948 * net hydrologic retention - 0.015 * inflow NO_3^--N concentration))^2; $R^2_{adj}=0.815$, $p<0.01$, $n=25$). Significant regression models were not obtained for TON-N and NH_4^+-N.

Table 4. Results of Spearman correlations between the retention efficiencies (%R) of the different nitrogen forms and the environmental factors by considering the dataset registered during the study period in both wetland-streams. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.

	NO_3^--N %R	TON-N %R	NH_4^+-N %R
	r	r	r
Inlet discharge ($l\ s^{-1}$)	-0.419*	-0.099	0.221
Net hydrologic retention	0.834**	0.429*	0.244
Inflow NO_3^--N concentration ($mg\ l^{-1}$)	-0.655**		
Inflow TON-N concentration ($mg\ l^{-1}$)		0.519**	
Inflow NH_4^+-N concentration ($mg\ l^{-1}$)			0.429*
Inflow NO_3^--N load ($g\ m^{-2}\ d^{-1}$)	-0.370		
Inflow TON-N load ($g\ m^{-2}\ d^{-1}$)		0.095	
Inflow NH_4^+-N load ($g\ m^{-2}\ d^{-1}$)			0.083
Water temperature (ºC)	0.256	-0.196	-0.258
Air temperature (ºC)	0.125	-0.146	-0.282

4 Discussion

4.1 Nitrogen retention efficiencies

This study shows that Mediterranean wetland-streams affected by agricultural inputs can remove efficiently TN-N from water. The retention efficiency was strongly influenced by N speciation in agreement with previous studies (Spieles and Mitsch 2000; Vellidis et al. 2003; Knox et al. 2008).

Wetland-streams have showed most efficient for removing NO_3^--N from water, the dominant N form, but were less efficient for the removal of TON-N and NH_4^+-N. The studied wetlands were sinks for TN-N and NO_3^--N during all the study period, while they were sources for TON-N and NH_4^+-N under some circumstances. Several studies have shown the ability of wetlands to remove NO_3^--N from water. Knox et al. (2008) found a mean retention efficiency for NO_3^--N of 60.0% in a natural flow-through wetland of California with a Mediterranean climate that collected agricultural runoff whose mean NO_3^--N concentration was 0.2 mg l^{-1}. Jordan et al. (2003) showed that a restored wetland removed 52.0% of the NO_3^--N received from agricultural runoff whose usual NO_3^--N concentration values were < 1 mg l^{-1}. In the studied wetland-streams, the mean retention efficiency for NO_3^--N (72.3%) was higher than that found in these aforementioned studies, even though the mean inflow concentration for NO_3^--N was above 20 mg l^{-1}. Besides, other studies performed in constructed wetlands generally show lower retention efficiencies for NO_3^--N than our results (Spieles and Mitsch 2000; Braskerud 2002; Mitsch et al. 2005). By considering both the annual mean inflow load of NO_3^--N and the annual mean retention efficiency, the Taray and Parra wetland-streams were capable of removing mean values of 1.6 and 0.8 kg of NO_3^--N a day^{-1}, respectively.

Denitrification, biological uptake and microbial immobilization are the main mechanisms for NO_3^--N removal in wetlands (Reddy and Patrick 1984; Bowden 1987; Groffman et al. 1992). These processes are influenced by the hydrologic conditions of wetlands (De Laune et al. 1981; Bowden 1987; Pinay et al. 2007). In the studied wetland-streams, NO_3^--N retention efficiency was negatively correlated with the inlet discharge and positively correlated with net hydrologic retention, thus suggesting that longer water residence times allow a longer time for NO_3^--N removal from surface water. Nutrient retention in wetlands is governed not only by changes in the hydrographs, but also by both the flow-through (velocity) and water residence time rates (Howard-Williams 1985). If water moves through a wetland at a quicker rate than that of N retention processes (denitrification or biological uptake), then considerable flow-through of N will take place. Peverly (1982) found that wetlands only retained nutrients when flow-through rates were low, while Stanley and Ward (1997) observed that net retention for all the N forms was strongly correlated with hydrological retention in the Talladega Wetland Ecosystem (TWE, Alabama, USA).

On the other hand, several authors have reported that denitrification may be potentially important in aquatic systems dominated by fine sediments, high NO_3^--N and organic carbon availability, a low redox potential of sediments, and warm water temperature (Faulkner and Richardson 1989; García-Ruiz et al. 1998; Inwood et al. 2007; Pinay et al. 2007). Unlike organic matter (and N) accumulation, which conserves N within the wetland, denitrification represents a permanent N loss from the system. Natural wetland sediments are chemically reduced and frequently contain ample organic carbon. Therefore, denitrification in wetlands is generally limited by nitrate availability (Ambus and Lowrance 1991). Nonetheless, this is not the case of the wetlands affected by agricultural inputs. Therefore, although denitrification was not estimated in the studied wetland-streams, this process is proposed to be an important pathway for NO_3^--

N loss because its occurrence is consistent with their environmental characteristics (high NO_3^--N availability, high water temperature, anoxic-black sediments and high hydrologic retention).

The retention capacity of wetlands varies seasonally, particularly in temperate regions where biological activity diminishes in winter (Howard-Williams 1985; Groffman et al. 1992). In fact, studies performed in these regions show that NO_3^--N retention efficiency is controlled mainly by the temperature (Spieles and Mitsch 2000; Chavan et al. 2008). In the studied wetland-streams, NO_3^--N retention efficiency tended to increase in summer months although significant differences among seasons were only observed in the Taray wetland (the lack of statistical significance for Parra wetland may be explained by the absence of data from July to September 2007, during the drought period). However, in contrast with the previously mentioned studies, we did not find correlation between NO_3^--N retention efficiency and temperature in the studied wetland-streams. One suggestion to explain the lack of correlation between both variables is that the warm temperate climate of the study area enables the continuous operation of the essential biogeochemical processes involved in NO_3^--N removal. This lack of correlation reinforces the fact that Mediterranean wetland-streams can significantly remove N input. We attributed the increase of NO_3^--N retention efficiency during the summer months to optimum hydrological conditions, as high net hydrologic retention rates, that favour N proccesing in wetlands (mainly biological uptake and denitrification). The increases of Cl^- concentrations during the summer months support the idea of evapotranspiration as the responsible factor of the hydrologic retention increases in the studied wetland-streams.

Wetland-streams acted as sinks for TON-N during most of the study period with net removal mean values of 153.9 and 34.4 g a day^{-1} for Taray and Parra wetlands, respectively. However, they were also sources for TON-N in some occasions. Similar results were obtained by other authors. For example, Jordan et al. (2003) reported TON-N retention efficiencies ranging from –15.0% to 39.0%.

TON-N retention in the studied wetland-streams, as same as in other wetlands, could be greater than the values obtained by input-output balance. Leaching and decomposition of autochthonous particulate organic matter is an additional source of organic N and decreases net TON-N retention. Decomposition of litter is probably the major source of TON-N in our wetland-streams, as other studies reported (Howard-Williams 1985; Bowden 1987; Chapman et al. 2001). In fact, some of these studies show that TON-N concentrations are generally higher in summer and fall and suggest increases relate to the autochthonous litter decomposition or to primary production. In contrast, no seasonal pattern was observed in our wetlands study. Bernal et al. (2005) also reported the absence of such a pattern in TON-N retention for an intermittent Mediterranean stream.

As same as previous studies (Braskerud 2002), TON-N retention efficiency was positively correlated with the inflow TON-N concentration. In addition, it was positively correlated with the net hydrologic retention, probably because the sedimentation of the organic matter associated with soil particles and the processing rates of TON-N to inorganic forms were higher under greater residence time of water within the wetlands (Jordan et al. 2003).

The studied wetland-streams were usually net sources of NH_4^+-N over the study period. However, when wetland-streams occasionally retained NH_4^+-N, their retention values were relatively high in comparison with those of previous studies. For example, Braskerud (2002) showed a mean retention value of 1.0% in small constructed wetlands that treat agricultural non-point source pollution. We suggest that litter decomposition and mineralization are the main autochthonous sources of NH_4^+-N in wetland-streams. Once wetland vegetation has died, a large and complex series of nutrient transformations emerges, all of which are associated with the leaching of detritus and simultaneous decomposition (Howard-Williams 1985). Several studies have demonstrated that plant detritus processing may be an important source of nutrients (Howarth and Fisher 1976; MacLean and Wein 1978). Kinetic mineralization of TON-N probably proceeds more rapidly than nitrification, thus NH_4^+-N concentration increases in surface water (Kadlec and Knight 1996; Braskerud 2002).

On the other hand, NH_4^+-N is more sensitive than NO_3^--N to slight changes of local conditions (chemical, physical and biological variables) (Hill 1996; Butturini and Sabater 1998; Gücker and Boëchat 2004), which also change as flow discharge does (Fisher et al. 1998; Von Schiller et al. 2008). Furthermore, NH_4^+-N reacts abiotically via adsorption/desorption reactions, and displays processing lengths that reflect the nature of the sediments and the chemical environment (Triska et al. 1994). Both properties are spatially heterogeneous in wetlands, and this variability increases as flow discharge decreases (Gücker and Boëchat 2004), which also occurs close to wetland-stream outlets. Thus, slight changes in the sediment redox potential may not only affect the exchange of NH_4^+-N at the water-sediment interface, but may also influence the

NH_4^+-N concentration in surface water (De Laune et al. 1981; Bowden 1987). The fact that NH_4^+-N retention efficiency was lower than that for NO_3^--N, and that it was even exported from wetland-streams, is consistent with this idea.

The temporal variability of the NH_4^+-N retention was very high in this study and was only positively correlated with the inflow NH_4^+-N concentration. However, Sabater et al. (2000) showed that 83.0% of the seasonal variation in the NH_4^+-N retention efficiency in a Mediterranean stream without riparian vegetation is explained by water temperature. The lack of correlation between other environmental factors and NH_4^+-N retention in the studied wetland-streams may be explained by the high sensitivity of NH_4^+-N concentration to slight changes of the local conditions (sediment redox potential, organic matter content, etc.) as we previously suggested.

4.2 Influence of the hydrologic retention and the inflow N concentration on the NO_3^--N retention efficiency

The main factors controlling the NO_3^--N retention efficiency in the studied wetland-streams are the hydrologic retention and the inflow NO_3^--N concentration. We hypothesized that a higher hydrologic retention increases NO_3^--N retention efficiency through an increase of biological processing rates (as biological uptake and denitrification).

Net hydrologic retention was used as an indirect measurement of the water residence time in wetland-streams. This factor often influences the N retention in aquatic systems because a longer contact time between surface water and sediment implies that the total amount of processed N increases (Peterson et al. 2001; Gücker and Boëchat 2004).

Inflow NO_3^--N concentration was the second factor controlling the NO_3^--N retention in the studied wetland-streams. Other studies in both, riparian buffers and natural/constructed wetlands, report a similar relationship between both variables (e.g., Spieles and Mitsch 2000; Sabater et al. 2003). In addition, these authors suggest a saturation effect by a high NO_3^--N load which exceeds the buffering capacity of these systems. Although the inflow NO_3^--N concentrations registered during the study period were high, they never exceeded the loading capacity of the wetland-streams, as high NO_3^--N retention rates indicated. Because almost 100 % of NO_3^--N retention efficiency is obtained with even high inflow NO_3^--N concentrations (e.g., 99.5 % with 21.4 mg l^{-1}), the retention efficiencies could not increase at lower inflow concentrations. Lower inflow NO_3^--N concentrations than 10.3 mg l^{-1} would be necessary to know the wetland-stream response to low N concentrations.

5 Conclusions

Our results emphasize the high efficiency of Mediterranean wetland-streams as N sinks all year round. This feature is influenced by low water discharges and probably, by the warm climate, both of which are key factors that make Mediterranean wetland-streams especially interesting compared to temperate wetlands. Wetland-streams consistently reduced NO_3^--N concentration, the dominant N form, throughout the year. They usually acted as sinks for TON-N and as sources for NH_4^+-N. Hydrological retention and inflow NO_3^--N concentrations were the main factors explaining the variability in NO_3^--N removal efficiency. However factors explaining TON-N and NH_4^+-N retention were not found. NO_3^--N retention showed a seasonal pattern but not directly associated with the temperature, but with the hydrological retention. The highest NO_3^--N retention effienciencies were detected during summer when evapotranspiration increased and as a consequence, wetland discharge decreased. Stream-wetlands showed a high effiency in N removal even at high N concentrations (21.4 mg l^{-1}). Our study in the Taray and Parra wetlands clearly demonstrates the crucial role of Mediterranean wetland-streams in the control of the N flux from agricultural landscapes to aquatic ecosystems located downstream.

The studied wetland-streams consistently reduce the N load, in such a way that the water leaving the wetlands is always of better quality than that entering them. In some countries, surface flow wetlands are highly valued for their high nutrient retention potential and their unique biodiversity. However, despite the high efficiency of the mediterranean wetland-streams to improve surface water quality, they are often desiccated for new agricultural purposes. Presently, there are an increasing number of activities aimed at restoring these sites as multifunctional landscape entities. In fact, there are studies which focus on identifying the most suitable areas for the restoration of surface flow wetlands to improve the water quality of a given catchment (Mitsch 1992). The wide distribution and strategic location of the Mediterranean wetland-streams in upstream reaches of basins makes them more interesting as special preservation ecosystems. Our results highlight the conservation interest of Mediterranean wetland-streams for two reasons, to protect wetland biodiversity and to improve the surface water quality in agricultural catchments in accordance with WFD’s objective (2000/60/EC).

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Chapter 2

Subsurface N retention in two Mediterranean wetland-streams affected by agricultural runoff

V García-García, R Gómez, MR Vidal-Abarca and ML Suárez

Department of Ecology and Hydrology, Faculty of Biology, University of Murcia, Campus of Espinardo, 30100 Murcia, Spain

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Abstract

We estimated subsurface nitrogen (N) retention efficiency of two Mediterranean wetland-streams affected by agricultural runoff, and analysed the influence of hydrology and physicochemical factors such as N load. Subsurface N retention efficiency was high and greater than the surface N retention quantified in a previous study. The studied wetlands clearly functioned as nitrate-N (NO_3^--N) sinks (96.5% and 89.8%), which was the dominant N form. They were usually total organic-N (TON-N) sinks (49.9% and 21.6%), but were ammonium-N (NH_4^+-N) sinks/sources. Water temperature was an important predictor of NO_3^--N retention efficiency, accounting for 59% and 34% of explained variance for both wetlands, respectively. This result highlights the importance of biological processes in NO_3^--N retention. The studied wetlands did not present "saturation symptoms" at high inflow NO_3^--N concentrations (40 mg l^{-1}). These small slope wetlands can be compared to riparian wetlands in terms of NO_3^--N retention efficiency. The contribution of the subsurface component to the wetland N mass removal rate was low (0.01% and 0.05%). Both wetlands removed an average of 1.1 and 6.2 μg of NO_3^--N m^{-2} h^{-1}.

1 Introduction

Excess nutrient loads have been recognised as the major cause of the serious water quality problems recently encountered in many estuaries and coastal waters around the world, as in surface waters and groundwaters. The USEPA considers nitrogen (N) to be one of the primary stressors in aquatic ecosystems (USEPA 2002). In this sense, the non-point-source pollution from agricultural areas has been recognised in many world regions to be the largest single source of nitrogen emission to aquatic environments (e.g., Carpenter et al. 1998; Birgand el at. 2007). Excess nitrogen is a pollutant that causes eutrophication in surface waters and contaminates groundwater, with subsequent environmental impacts such as toxic algal blooms, oxygen depletion, fish kills and loss of biodiversity (Vitousek et al. 1997).

Nitrogen concentration increases and hydrological regime changes as a result of agricultural development, and both are important impacts on aquatic ecosystems in the Mediterranean region (Pinay et al. 2002; Gómez et al. 2005), especially in the most arid zones where reduced surface flows diminish streams' dilution capacity.

Studies conducted across a wide geographic range have evidenced wetlands' capacity to reduce the N flux from terrestrial to aquatic ecosystems (e.g., Lowrance et al. 1984; Groffman et al. 1992; Sabater et al. 2003; Fisher and Acreman 2004). Thus, they become natural tools in the control of non-point source pollution at the catchment scale (e.g., Mitsch 1992; Mitsch et al. 2005). However, wetlands' capacity to retain N can vastly vary according to their biological, hydrological and physicochemical characteristics (e.g., Mitsch and Gosselink 1993; Mitsch et al. 2000).

In sedimentary rock dominated catchments from southeast (SE) of Spain, small wetland-streams are widely distributed. According to both wetlands landscape location and hydrology, these wetlands can be defined more specifically as slope wetlands (Stein et al. 2004; Acreman 2005). They are associated with alluvial/colluvial deposits and exist where topographic conditions allow subsurface water to intersect the soil surface, creating a zone of perennial or near-perennial moisture. Despite the aridity of this Mediterranean region, the predominant land use in many of these sedimentary catchments is irrigated agriculture supported by external water resources. Agriculture-derived water moves longitudinally through these wetlands before reaching the streams and reservoirs located down slopes. The results of a previous study in these wetlands (García-García et al. 2009) showed annual average N retention efficiencies for surface water of 87.9% and 50.9%. Nevertheless, surface flow is scarce in many of these wetlands, or is even absent. In these cases, agriculture-derived water flows exclusively through the wetland subsurface component. As there are differences in their physical structure, and in the biological and chemical characteristics between the surface *vs.* subsurface wetland components, substantial differences in N retention efficiencies may result from both components (e.g., Mayer et al. 2007). However, information about surface and subsurface N retention is required to fully understand the role of these small wetlands in controlling non-point source pollution at the catchment scale. This is an urgent need if we consider that these wetlands are one of the most threatened aquatic ecosystems in the southeast of Spain, mainly by large fertile soil demands for agricultural use.

Despite the widespread distribution of slope wetlands in southeast (SE) of Spain, very low information exist on their functioning, such that even no studies provide information on the N retention dynamic in their subsurface component. In fact, the literature is dominated by studies conducted in constructed wetlands or natural riparian wetlands. Thus, the data compiled from this study extends knowledge on Mediterranean wetlands functioning.

In agricultural Mediterranean areas, highly variable hydrological conditions related to the natural hydrological intermittency of Mediterranean aquatic systems (Gasith and Resh 1999; Gómez et al. 2009) and to agriculture-derived water inputs may act as a stress factor to N retention. Fluctuating hydrological conditions affect wetland hydrological parameters, such as hydraulic gradient and water table level, which both affect the water residence time and sediment redox conditions. These factors have been shown to influence the rate and type of the biogeochemical process taking place (Groffman et al. 1992; Simmons et al. 1992; Dahm et al. 1998; Sabater et al. 2003; Hefting et al. 2004). Besides hydrology, the extent (time duration) and magnitude (N load) of agricultural N inputs may also affect N retention in wetlands (Howard-Williams 1985). In fact, studies conducted in riparian wetlands have shown that chronic N inputs may result in N retention saturation (Groffman et al. 1992; Hanson et al. 1994; Sabater et al. 2003).

In this paper, we report the subsurface N retention efficiencies of two Mediterranean slope wetlands receiving non-point agricultural runoff inputs with high N loads. The specific objectives were to: i) quantify subsurface retention efficiency for nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), total organic nitrogen-N (TON-N) and total nitrogen-N (TN-N) over an annual cycle and to ii) examine the effect of hydrology and N load on subsurface N retention efficiency.

2 Materials and methods

2.1 Study site

The study sites, the Taray (0.5 ha) and Parra (0.7 ha) wetlands, are located at 211 m and 181 m above sea level, respectively, within the catchments of the Salada (Taray wetland) and Parra streams (Parra wetland) (the Murcia Region, southeast of Spain, 38º 9’ 26’’ N, 1º 9’ 4’’ W; 38º 13’ 93’’ N, 1º 4’ 23’’ W) (Fig. 1a). The climate in this region is semi-arid Mediterranean, and average annual precipitation and temperature are close to 300 mm and 18ºC, respectively (the Fortuna and Abanilla meteorological stations). Geology, vegetation and land use of wetland catchments, along with the vegetation and geomorphological features in these wetlands, are described in García-García et al. (2009). Wetland soils include Hyposodic Hypogypsic Gypsisols and Hypergypsic Gleyc Solonchaks (FAO 1998), which have a silt loam to clay loam texture and high salinity. The plant community is dominated by reed (*Phragmites australis*) and halophytic shrubs, such as *Suaeda vera*, *Sarcocornia fruticosa* and *Arthrocnemun macrostachium*, with a total cover of approx. 95% of the wetland surface. The wetlands under study and the adjacent agricultural uplands are hydrologically linked by subsurface water (Fig. 1b), and occasionally by overland water inputs when storm events occur. Therefore, the wetlands almost entirely receive water from subsurface water seepages located in the upper part (no additional substantial groundwater inputs were detected during the study period), minor inputs deriving from direct precipitation, and sporadic inputs of overland water flow resulting from storms (Fig. 1b). Subsurface water flows longitudinally through the wetlands (downslope) before typically discharging into small stream channels at the wetland outlet (Fig. 1b). Whereas the surface wetland discharge is usually low, evapotranspiration is an important water output pathway from the wetlands.

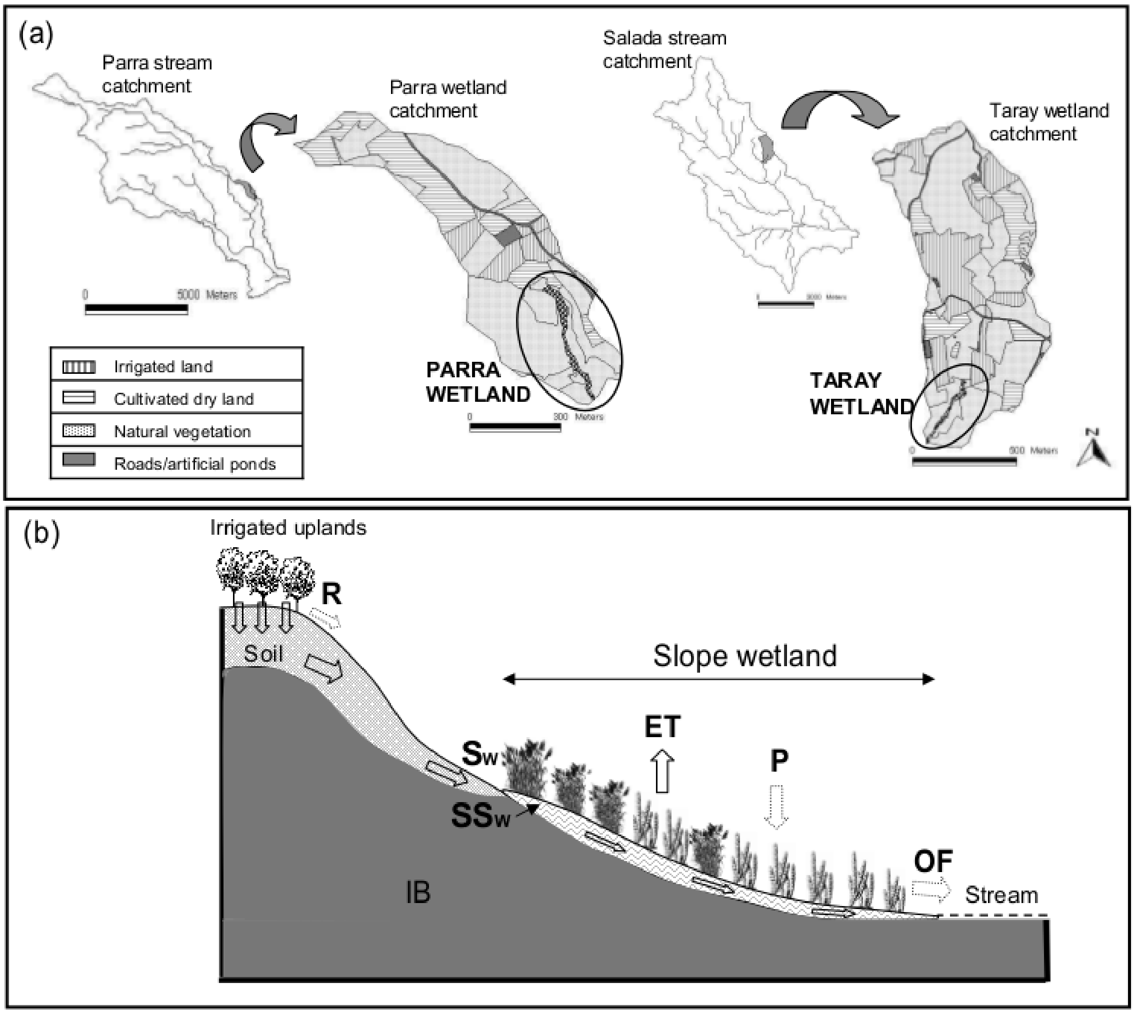

Fig. 1. **a** Location of the studied wetlands and their catchments. **b** Vertical section view of the studied wetlands showing the ways in which water can move into them or from them: *R* runoff water, *SW* surface water, *SSW* subsurface water, *IB* impermeable bed, *ET* evapotranspiration, *P* precipitation, *OF* surface and subsurface outflow to the river.

2.2 Field sampling

In each wetland, four sampling transects of piezometers were placed perpendicularly to the direction of the subsurface flow at 100 m intervals (Fig. 2). The first transect (T1) was located on the upsloping edge of the wetland near agricultural fields, and the last one (T4) was located at a distance of 300 m. In each transect, the number of piezometers varied from two to six depending on wetland width, which ranged from 3.4 m to 7.1 m in the Taray wetland, and from 2.3 m to 13.4 m in the Parra wetland. There were 14 and 19 piezometers in the Taray and Parra wetlands, respectively (Fig. 2). The piezometers along each transect were spaced at 1-metre intervals and were also inserted below the sediment surface at a depth of approximately 1 m. They were made from PVC pipes (i.d. of 2.96 cm) and were perforated with 0.4 cm holes in the lower 70 cm. The top and bottom of the pipe were sealed with PVC caps to avoid ingress of rainwater and sediment, respectively.

Piezometers were sampled once a month from March 2007 to March 2008 in the Taray wetland, and from April 2007 to June 2008 in the Parra wetland (the Parra wetland was dry from July to September 2007). On each sampling date, subsurface water samples were collected from the piezometers using a hand-operated peristaltic pump (Eijkelkamp Agrisearch Equipment B.V., Giesbeek, the Netherlands). Prior to piezometer sampling, the water table level was measured (Sounding apparatus, Eijkelkamp Agrisearch Equipment B.V., Giesbeek, the Netherlands). Three piezometer volumes were purged before to obtain a valid subsurface water sample. Water samples were stored in acid-washed polyethylene bottles inside coolers with block ice at 4ºC until they were transported to and analysed in the laboratory. Positive values refer to situations when the water table was above the ground surface (there was a surface water sheet on the ground), while negative values refer to situations when the water table was below the ground surface. Temperature, salinity and conductivity (Tetracon 325conductivity meter; WTW, Munich, Germany) were also recorded in each piezometer.

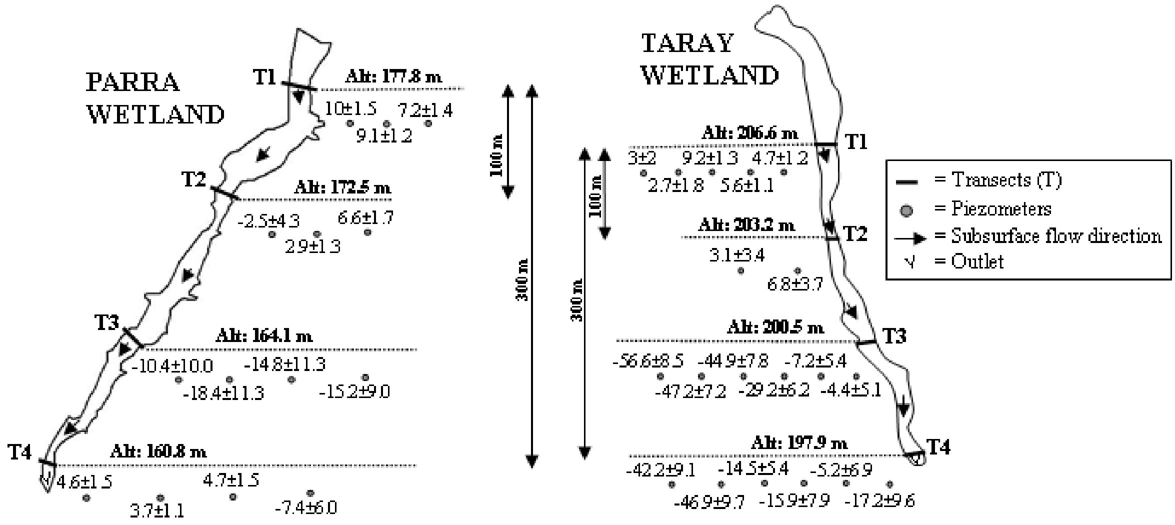

Fig. 2. Longitudinal view of the studied wetlands. The water table levels (mean ± SD) recorded in the wetland piezometers throughout the study period are shown (n=13 and n=12 for each piezometer in the Taray and Parra wetlands, respectively). Alt indicates the altitude of each transect.

2.3 Chemical analyses

The dissolved inorganic nitrogen (DIN) forms were analysed on filtered water samples (Whatman GF/F, 0.7 µm nominal pore size; Whatman International Ltd., Maidstone, England) within 24 h of collection. The total nitrogen (TN-N) analyses were performed on unfiltered and frozen water samples. The nitrate-N concentration (NO_3^--N) was measured by a colorimetric method following cadmium reduction to nitrite-N (NO_2^--N) (Wood et al. 1967). The NO_2^--N concentration was analysed by the diazotization method (Strickland and Parsons 1972). The NO_3^--N concentration was finally estimated by subtracting the NO_2^--N concentration obtained by diazotization. The ammonium-N concentration (NH_4^+-N) was measured by the phenol-hypochlorite colorimetric method (Solorzano 1969). DIN concentration was calculated as the sum of the NO_3^--N, NO_2^--N and NH_4^+-N concentrations. Total nitrogen (TN-N) samples were digested with potassium persulphate (D'Elia et al. 1977) and the resulting NO_3^--N was analysed by cadmium reduction using an automated ion analyzer (EasyChem Plus, Systea Analytical Technologies, Italy). The total organic nitrogen (TON-N) concentration was estimated by subtracting the DIN concentration from the TN-N concentration. The chloride concentration (Cl^-), used as a passive tracer, was analysed on filtered water samples (Whatman GF/F) within 48 h of collection by the silver nitrate volumetric method (APHA 1985).

2.4 Subsurface wetland discharge calculations

Based on previous studies (e.g., Lowrance et al. 1984; Jordan et al. 1993; Sabater et al. 2003), Darcy's equation (Eq. 1) was used to estimate the subsurface discharge entering the study wetlands:

$$Q = K \times I \times A \qquad (1)$$

where Q is the water discharge (m^3 h^{-1}), K is saturated hydraulic conductivity (m h^{-1}), I is the water table slope or the hydraulic gradient (m m^{-1}), and A is the saturated area (m^2). Hydraulic conductivity was estimated to be 3.6 x 10^{-4} m h^{-1} based on the hydraulic conductivity data for clay loam soils at saturation (Klute and Dirksen 1986). The hydraulic gradient was calculated as the difference of the mean water table levels between the upper or inlet transect (T1) and the lower or outlet transect (T4), divided by the distance between both transects (300 m). The saturated area was estimated by multiplying the saturated depth by a width of 1 m to compare the unit loads among the piezometers (Lowrance et al. 1984; Sabater et al. 2003). Saturated depth was estimated by assuming that each soil profile conducted water through the top 1 m (Gómez 1995).

2.5 N retention calculations

To develop an N budget for the subsurface component of the studied wetlands, we used a simple hydrologic balance (SSW_{in} = ET + SSW_{out}) in which the important water inputs and outputs were identified: SSW_{in} is the subsurface discharge in the upper transect (T1), ET is the evapotranspiration rate and SSW_{out} is the subsurface discharge in the lower transect (T4). From the spatial variability data of the subsurface water Cl^- concentration and the water table levels, we assumed that no additional substantial hydrological inputs and outputs occurred. Cl^- was used as a passive tracer to detect the dilution or concentration (by evapotranspiration) of the solutes in the subsurface water between the inlet (T1) and outlet (T4) transects (Fig. 2) (Simmons et al. 1992; Sabater et al. 2003). The Cl^- concentration was also used to correct the effect of evapotranspiration or any potential dilution effect on solute concentrations (Eq. 2). Despite the vertical water exchanges between surface and subsurface components being made possible through wetland transport, we assumed that dilution by surface water was irrelevant and did not affect the N concentrations. This assumption is supported by the fact that the subsurface water Cl^- concentrations were higher than those from the surface and that the Cl^- concentrations in the outlet piezometers were always higher than in the inlet piezometers. Therefore, the retention efficiency (%R) for the different N forms (NO_3^--N, NH_4^+-N, TN-N and TON-N) was calculated by considering Eq. 2 (Trudell et al. 1986):

$$\%R = (1- (N/Cl^-_{out} / N/Cl^-_{in})) \times 100 \qquad (2)$$

where %R is the percentage of N removed by the wetlands in relation to inflowing N, and N/Cl^-_{out} / N/Cl^-_{in} are the concentration ratios of both solutes in the outlet (T4) and inlet (T1) piezometer transects, respectively. A positive %R indicates that the wetland was an N sink and, conversely, a negative value indicates that the wetland was an N source. The inflow N load (mg N m^{-2} h^{-1}) on each sampling date was calculated as the product of the mean inflow N concentration (mg l^{-1}) obtained from the piezometers located in T1 by the subsurface discharge (l m^{-2} h^{-1}) at this point. The non-retained N percentage (100 - %R) was applied to the inflow N load to estimate the outflow N load (mg N m^{-2} h^{-1}). Net N removal was calculated as the difference between the inflow and the outflow N load. The retained N load could be slightly overestimated or underestimated by the use of a literature value for hydraulic conductivity to estimate subsurface discharge. However, it does not affect the wetland retention efficiency (%R), which is the main variable analysed in this study.

2.6 Statistical analyses

Paired t-tests separately for each wetland were used for detecting significant differences between the inflow and outflow N concentrations. When it was necessary, the inflow and outflow N concentration data were log(x+1)-transformed to ensure normality and homogeneity of variance prior to statistical analyse. We also used the Wilcoxon signed-rank tests to detect significant differences between the inflow and outflow N concentrations when data could not be assumed to be normally distributed, even after transformation. The coefficient of variation (%CV) was used to quantify the annual variability of the physicochemical parameters and wetland N retention efficiency (%R). Pearson product-moment correlation analyses separately for each wetland were conducted to explore the relationship between N retention efficiency and the physicochemical/hydrological parameters. To analyse the seasonal differences in the N retention efficiency for each study wetland, months were grouped as follows: spring (March, April and May), summer (June, July, August and September), autumn (October and November) and winter (December, January and February). Considering the available data, seasonal differences were tested using a one-way ANOVA with a Tukey's HSD post-hoc test. Linear regressions analyses were used to explain N retention efficiency in each wetland separately. In order to bind the limits of prediction by linear regressions into bounds 0-1 (0-100%), as the use of the next expression $(1-(N/Cl^{-}_{out} / N/Cl^{-}_{in})) \times 100$ (Eq. 2) may result in an unrealistic case of regression predicting > 100% retention, we decided to use the next highly correlated ($p<0.01$)

function, ln (N/Cl^-_{in} / N/Cl^-_{out}), for the statistical analyses (correlation analyses, one-way ANOVA and linear regressions). Shapiro-Wilk and Levene's tests were applied to test normality and homogeneity of variance, respectively. Statistical analyses were done with SPSS (SPSS software version 15.0.1 for Windows; SPSS Incorporated, Chicago, Illinois).

3 Results

3.1 Hydrological and physicochemical characterisation of subsurface flow

The water table level showed the same trend in both wetlands: higher water levels in spring and autumn, and lower levels in summer. However, whereas the water table level was generally above the sediment surface (positive values) in the Taray wetland, it was usually below the sediment surface (negative values) in the Parra wetland. The annual average of the subsurface inflow was low, but was 3 times lower in Taray (0.0001 ± 0.0001 l m^{-2} h^{-1}, n=13) than in Parra (0.0003 ± 0.0001 l m^{-2} h^{-1}, n=12) (Table 1). The annual variability of the subsurface inflow was high, 69% (n=13) and 49.7% (n=12) in the Taray and Parra wetlands, respectively. The hydraulic gradient was low in both wetlands, with maximum values observed in summer (Table 1).

The annual average subsurface water temperature was high in both wetlands (Table 1). Despite the annual variation of water temperature being relatively low, 28.1%, (n=13) and 15.3% (n=12) in the Taray and Parra wetlands, respectively, the average water temperature in summer increased by about 8ºC and 4ºC in each wetland, respectively. In summer, the water temperature was 22.9 ± 0.9ºC (n=4) and 19.5 ± 0.8ºC (n=2) in the Taray and Parra wetlands, respectively. In winter, it was 12.4 ± 0.4ºC (n=3) in the Taray and 13.3 ± 1.1ºC (n=3) in the Parra wetland.

Table 1. Mean, maximum and minimum values of the different hydrological and physicochemical parameters measured in the subsurface water of both study wetlands during the study period (n=13 and n=12 in the Taray and Parra wetlands, respectively). [a] Negative values indicate the water table level under the ground surface.

	Taray wetland			Parra wetland		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Water table level (cm)[a]	-1.6	12.7	-27.9	-12.1	2.7	-24.9
Subsurface discharge (l m^{-2} h^{-1})	0.0001	0.0003	0.00002	0.0003	0.0006	0.0001
Hydraulic gradient (m m^{-1})	0.0002	0.0007	0.00007	0.0008	0.0017	0.0004
Water temperature (ºC)	17.2	23.8	10.2	16.1	20	12
Water conductivity (mS cm^{-1})	26.5	29.4	22.8	17.9	21	16

The water Cl^- concentration was high in both wetlands, and the outflow Cl^- concentrations were always higher than the inflow Cl^- concentrations (Fig. 3). The annual variation of the inflow Cl^- concentration was greater in the Parra wetland (CV=24.2%, n=12) than in Taray (CV=5.1%, n=13). Reductions in the inflow Cl^- concentration generally coincided with the increased inflow NO_3^--N concentration in the Parra wetland (Fig. 3); this pattern was not observed in the Taray wetland. The highest Cl^- concentrations were observed in both wetlands in summer and early autumn, except for the dry months in the Parra wetland (Fig. 3).

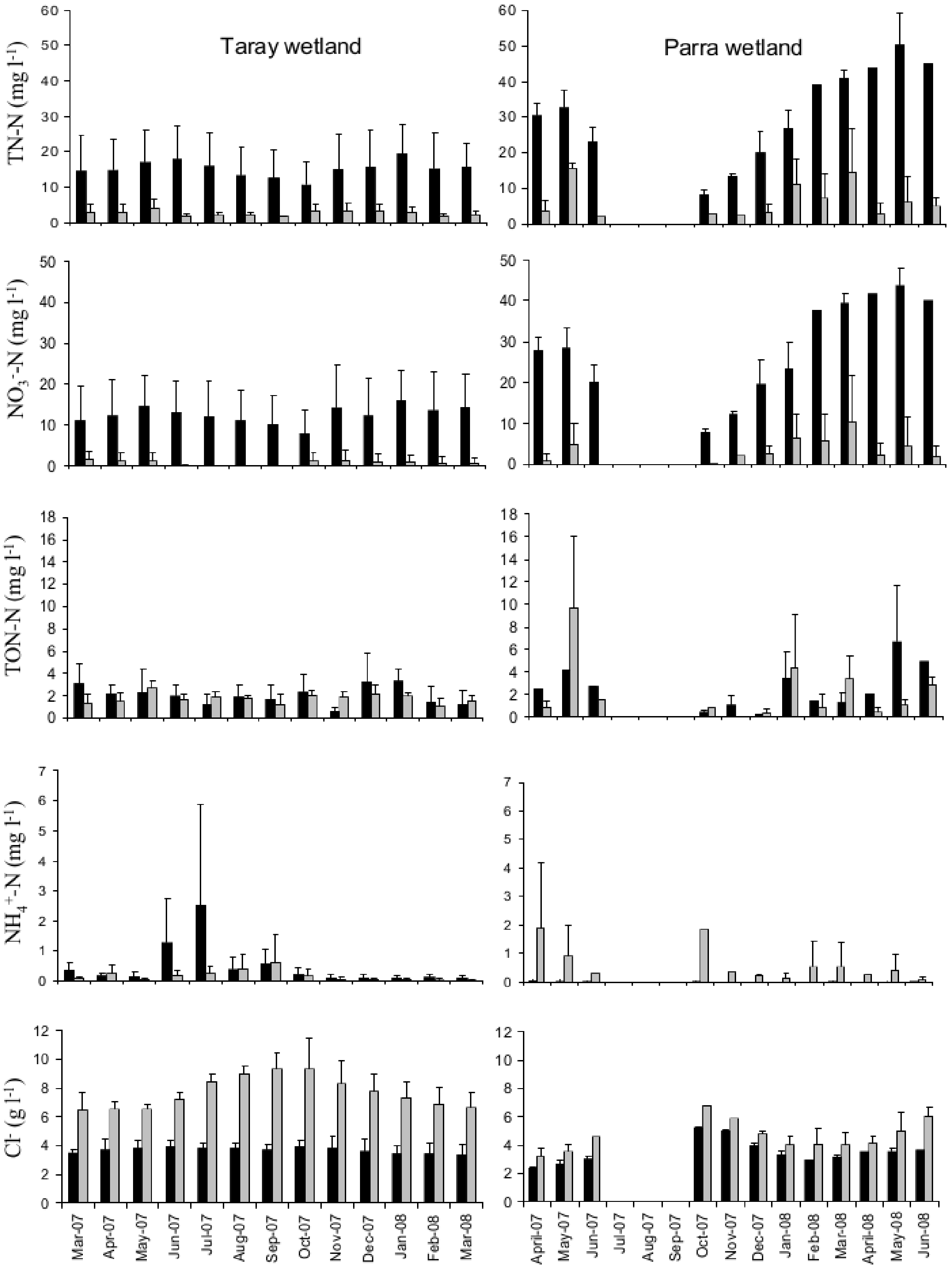

Fig. 3. Annual variation of the inflow (black bars) and the outflow (grey bars) TN-N, NO_3^--N, TON-N, NH_4^+-N and Cl^- concentrations (mean + SD) for the Taray (left) and the Parra (right) wetlands. Lack of data in some months for the Parra wetland indicates that it was dry.

The average inflow TN-N concentration was high in both wetlands, but was twice as high in Parra than in Taray (Table 2). NO_3^--N was the dominant N form in the inflow water of both wetlands (82.2% and 91.5% in Taray and Parra, respectively), followed by TON-N and NH_4^+-N (Table 2). Similarly to the Cl^- concentration, the annual variability of the inflow NO_3^--N concentration was greater in Parra (CV=42.4%, n=12) than in Taray (CV=17.4%, n=13), with a more stable inflow NO_3^--N concentration over time (Fig. 3). After the dry period in the Parra wetland, the inflow NO_3^--N concentration sharply increased from October to February 2008. The annual variability of the inflow TON-N and NH_4^+-N concentrations was more marked than for NO_3^--N, showing no seasonal trends (Fig. 3). The highest annual %CV for both N forms occurred in the Parra wetland. %CV of 41.2% (n=13) and 74.9% (n=12) for TON-N, and 49.1% (n=13) and 84.6% (n=11) for NH_4^+-N were observed in the Taray and Parra wetlands, respectively.

3.2 Nitrogen retention efficiencies

The inflow and outflow N loads, along with N retention efficiencies, are shown in Table 2. The Parra wetland exhibited the highest inflow and outflow N loads, except NH_4^+-N inflow load, which was higher in the Taray wetland. The inflow and outflow concentrations for every N forms were significantly different in both wetlands (Table 2). Total N retention efficiencies ranged from 85.7% to 94.6% in Taray and from 62.1% to 94.4% in Parra. NO_3^--N was the N form with the highest retention efficiencies (ranging from 91.4% to 100% in Taray and from 76.0% to 99.7% in Parra). NO_3^--N retention efficiency increased significantly in summer months (June-September) in

Taray (one-way ANOVA, $F_{3,9}=39.8$, $p<0.05$). NO_3^--N retention efficiency also increased slightly in June 2007 and June 2008 in the Parra wetland; however this wetland was dry for the remaining summer months. Consequently, the data available in the Parra wetland did not suffice to obtain significant interseason differences.

Table 2. Concentration, load, net retention, retention efficiency (%R) and the annual coefficient of variation for %R (%CV) of the different N forms and Cl^- measured in the subsurface water of the studied wetlands during the study period. Values are the mean ± SD (n=13 and n=12 in the Taray and Parra wetlands, respectively). Significant differences between inflow and outflow concentrations for the different N forms are indicated by ** $p < 0.01$ (paired t-test or Wilcoxon signed-rank test). The retention efficiency (%R) values are calculated from the mean values of the N/Cl^- ratio for T1 and T4 following Eq. 2 (see the N retention calculation section).

	Concentration (mg l^{-1})		Load (µg m^{-2} h^{-1})		Net retention (µg m^{-2} h^{-1})	%R	
	Inflow	Outflow	Inflow	Outflow		mean ± SD	%CV
Taray wetland							
TN-N	15.2 ± 2.3	2.7 ± 0.7**	1.32 ± 0.94	0.1 ± 0.06	1.22 ± 0.89	91.3 ± 3.1	3.4
NO_3^--N	12.5 ± 2.2	0.8 ± 0.6**	1.08 ± 0.71	0.03 ± 0.03	1.05 ± 0.71	96.5 ± 2.9	3.0
NH_4^+-N	0.5 ± 0.7	0.2 ± 0.2**	0.07 ± 0.18	0.01 ± 0.01	0.06 ± 0.17	73.0 ± 23.5	32.2
TON-N	2.0 ± 0.8	1.7 ± 0.4**	0.16 ± 0.1	0.07 ± 0.05	0.09 ± 0.08	49.9 ± 33.2	66.6
Cl^-	3.7 ± 0.2	7.7 ± 1.1	_	_	_	_	_
Parra wetland							
TN-N	31.2 ± 13.3	6.4 ± 4.8**	7.71 ± 3.8	1.31 ± 1.37	6.4 ± 3.3	82.2 ± 11.6	14.1
NO_3^--N	28.5± 12.1	3.5 ± 3.1**	7.0 ± 3.25	0.66 ± 0.56	6.3 ± 3.1	89.8 ± 8.2	9.1
NH_4^+-N	0.02± 0.01	0.63 ± 0.6**	0.01± 0.01	0.15 ± 0.17	-0.14 ± 0.16	-3941.5± 4132.8	104.9
TON-N	2.6 ± 1.9	2.2 ± 2.7**	0.69± 0.62	0.51 ± 0.9	0.18 ± 0.68	21.6 ± 73.8	341.8
Cl^-	3.5 ± 0.9	4.7 ± 1.1	_	_	_	_	_

Ammonium-N retention efficiency differed substantially between both wetlands (Table 2). The Taray wetland generally exhibited high NH_4^+-N retention efficiencies, ranging from 3.0% to 95.6%, whereas the Parra wetland always displayed negative values, denoting NH_4^+-N exportation. No seasonal trend was observed in either wetland.

TON-N retention efficiency was usually positive in both study wetlands, although negative values were occasionally recorded. No seasonal pattern was detected despite strong fluctuations in both wetlands over time.

The TN-N net load (mg m^{-2} h^{-1}) removed from the subsurface water was low despite the high TN-N retention efficiencies of both study wetlands (Table 2). Although the highest retention efficiencies for each N form were found in the Taray wetland, the mass removal rate (mg m^{-2} h^{-1}) was higher in the Parra wetland, where higher N loads were detected (Table 2).

3.3 Relationships between N retention efficiency and hydrological/physicochemical factors

NO_3^--N retention efficiency was significantly and positively correlated with the subsurface water temperature in both wetlands (Table 3). Besides, it was positively correlated with the hydraulic gradient and the subsurface discharge in both wetlands (Table 3). Both hydrological variables showed their highest values during the summer months coinciding with the highest water temperatures. NO_3^--N retention efficiency was also negatively correlated with the water table level in the Taray wetland. Finally, the regression analyses indicated that 59% and 34% of variance in NO_3^--N retention efficiency was explained by the subsurface water temperature in both wetlands (R^2=0.591, $p<0.01$, n=13 in the Taray wetland and R^2=0.335, $p<0.05$, n=12 in the Parra wetland). No significant relationship was found between the NO_3^--N retention efficiency and the hydrological variables.

NH_4^+-N retention efficiency was only significantly and positively correlated with the inflow NH_4^+-N concentration in the Taray wetland (Table 3). The regression analyses indicated that the inflow NH_4^+-N concentration explained 49% of variance in NH_4^+-N retention efficiency in this wetland (R^2=0.489, $p<0.01$, n=13).

TON-N only showed a significant positive correlation with the inflow TON-N concentration in the Taray wetland (Table 3). TN-N showed no relevant relationships with the studied hydrological and physicochemical variables (Table 3).

Table 3. Pearson product-moment correlation coefficients for the retention efficiency of the different N forms and the environmental factors for the data set measured during the study period in each wetland. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.

	Taray wetland				Parra wetland			
	TN-N	NO_3^--N	NH_4^+-N	TON-N	TN-N	NO_3^--N	NH_4^+-N	TON-N
Water temperature	0.348	0.769**	0.121	-0.219	0.367	0.599*	0.187	0.035
Water table level	-0.609*	-0.925**	-0.060	0.083	-0.034	-0.382	0.494	-0.127
Hydraulic gradient	0.464	0.620*	0.470	-0.209	0.090	0.640*	-0.460	-0.150
Subsurface discharge	0.463	0.613*	0.465	-0.207	0.091	0.639*	-0.460	-0.149
Inflow TN-N concentration	0.282	-0.130	0.409	-0.090	0.311	-0.230	0.629*	-0.149
Inflow NO_3^--N concentration	0.193	-0.261	0.182	-0.327	0.307	-0.255	0.611*	-0.167
Inflow TON-N concentration	-0.330	-0.331	-0.122	0.772**	0.106	0.007	0.534	0.016
Inflow NH_4^+-N concentration	0.488	0.603*	0.699**	-0.210	0.205	0.384	0.391	-0.197
Inflow TN-N load	0.459	0.549	0.549	-0.251	0.264	0.328	0.163	-0.254
Inflow NO_3^--N load	0.471	0.537	0.521	-0.313	0.274	0.311	0.152	-0.279
Inflow TON-N load	0.181	0.345	0.254	0.369	0.173	0.355	0.219	-0.096
Inflow NH_4^+-N load	0.380	0.501	0.626*	-0.274	-0.021	-0.177	0.094	-0.056

4 Discussion

This study reveals a high subsurface NO_3^--N retention efficiency (the dominant N form) in both wetlands, which is even higher than the estimated surface retention efficiency (García-García et al. 2009). A comparable pattern is observed in other similar operational wetlands, such as riparian wetlands (Mayer et al. 2007). In relation to the surface component, greater hydrological retention in the subsurface system results in a longer water residence time, which enhances the interaction between subsurface water and biologically active sediments. Ecosystem retention is the product of the biological processing rate and the water residence time (Valett et al. 1996), resulting in greater N retention efficiencies as the residence time prolongs (e.g., Groffman et al. 1992; Clément et al. 2003). However, subsurface retention efficiency markedly differed among N species (Table 2), as previous studies have reported (e.g., Schade et al. 2002; Vellidis et al. 2003). The greatest subsurface retention efficiency was seen for NO_3^--N, as formerly described for surface retention (García-García et al. 2009). Studies into riparian wetlands have also reported higher retention efficiencies for NO_3^--N (over 80%) than for NH_4^+-N (e.g., Lowrance et al. 1984; Jordan et al. 1993). These results suggest that these small slope wetlands may be comparable to riparian wetlands in terms of their efficiency to control non-point source pollution. In both the Taray and Parra wetlands, the annual average subsurface NO_3^--N net retention rate depended on inflow loading, and was 0.1 kg ha^{-1} yr^{-1} and 0.6 kg ha^{-1} yr^{-1}, respectively.

Denitrification, plant uptake and microbial immobilisation are the main NO_3^--N retention processes (Reddy and Patrick 1984; Bowden 1987; Groffman et al. 1992), whereas mineralisation of organic matter and its subsequent nitrification can result in NO_3^--N release (Schade et al. 2002). As NO_3^--N synthesis is limited under dominant anaerobic conditions, which prevails in the subsurface component, greater net retention efficiency is expected than for the surface component. The lower temporal variability of subsurface net NO_3^--N retention efficiency as compared to the surface component (García-García et al. 2009) may, in part, be also explained by this fact.

Although denitrification was not estimated, we suggest that this process, together with plant uptake, plays an important role in NO_3^--N retention. High denitrification rates are expected in aquatic systems with fine-texture sediments and high NO_3^--N and organic carbon availability (Triska et al. 1984; Hill and Sanmugadas 1985; Groffman and Tiedje 1989; Rivett et al. 2008; James 2010). The fact that water temperature is an important predictor for NO_3^--N retention efficiency in the wetlands under study (accounting for 59% and 34% of explained variance), it highlights the importance of the biological processes in NO_3^--N retention, and this result is consistent with previous studies (e.g., Lowrance et al. 1984; Pinay and Décamps 1988; Pinay et al. 1993; Saunders and Kalff 2001; Rivett et al. 2008; Chavan et al. 2008). Nevertheless, this positive relationship between water temperature and NO_3^--N retention efficiency may also be driven by a prolonged water residence time in summer (e.g., Groffman et al. 1992; Clément et al. 2003), when the highest water temperatures were reached. Despite hydraulic gradient usually affects negatively the NO_3^--N retention efficiency (e.g., Simmons et al. 1992; Cirmo and McDonnell 1997; Sabater et al. 2003; Hefting et al. 2004; Pinay et al. 2007), we found no such relationship. The low hydraulic gradients of the studied wetlands (Table 1) likely provide suitable conditions (long water residence times) to enable the processes involved in NO_3^--N retention to operate continuously.

The wetlands' ability to lower water NO_3^--N concentration may diminish over time if they receive high NO_3^--N inputs for many years because microbial and plant sinks may become "N-saturated" (Groffman et al. 1992; Hanson et al. 1994). However, the studied wetlands maintained high subsurface NO_3^--N retention efficiencies (> 97%), even at high inflow NO_3^--N concentrations (40 mg l^{-1}). This result seems to indicate that NO_3^--N retention processes are not overloaded, and reinforces the assumption that continuous favourable environmental conditions in a Mediterranean climate allow high NO_3^--N retention, even under high N loads. Previous studies in wetlands neither found NO_3^--N processing saturation at high NO_3^--N concentrations (Cooper 1990; Brin et al. 2010).

The study wetlands acted very differently in terms of NH_4^+-N retention. The Taray wetland tended to act as an NH_4^+-N sink, while the Parra wetland acted as an NH_4^+-N source to downstream waters (Table 2). The main processes involved in NH_4^+-N removal are biological uptake, nitrification and adsorption on organic matter and the clay cation exchange complex (Howard-Williams 1985). The last two processes are directly influenced by sediment oxygen content (redox potential). Thus, whereas under highly reducing environments, like those occurring in flooded environments, nitrification is inhibited (it occurs mainly in environments with a high redox potential value), the dissimilatory NO_3^--N reduction to NH_4^+-N and the NH_4^+-N release from the organic matter/clay cation exchange complex generally exceed NH_4^+-N removal processes (Tiedje et al. 1982; Silver et al. 2001). Although the sediment redox potential was not measured, differences in the sediment redox conditions between both wetlands might explain the differences observed in NH_4^+-N retention efficiency. This assumption is consistent with previous studies showing that NH_4^+-N retention depends mainly on sediment redox conditions (Lowrance et al. 1984; Mulholland 1992; Cirmo and McDonnell 1997; Young and Briggs 2005). On the other hand, the mineralisation of organic N is the main source of NH_4^+-N, whose ambient concentration may increase if nitrification is inhibited (e.g., Mulholland 1992; Hefting et al. 2004; Hoffman et al. 2006). Finally, Gücker and Böechat (2004) underlined the importance of the water residence time on NH_4^+-N retention efficiency which could also explain the differences observed in NH_4^+-N retention efficiency between both wetlands.

Subsurface TON-N retention efficiency was relatively high in both study wetlands (average values were 49.9% and 21.6% in the Taray and Parra wetlands, respectively), and was higher and less variable over time than surface retention efficiency (García-García et al. 2009). In contrast, riparian wetlands usually act as organic N sources (e.g., Lowrance et al. 1984; Schade et al. 2002; Pellerin et al. 2004). In the subsurface component of the study wetlands, N mineralisation and adsorption onto sediment particles, the main biotic/abiotic processes involved in water TON-N retention, might be favoured by longer water residence times (e.g., Hammer and Knight 1994; Jordan et al. 2003; Chavan et al. 2008). Besides, lower particulate organic matter inputs to the subsurface component might also explain their greater net TON-N retention efficiencies. Although some studies have reported higher mineralisation rates in summer (e.g., Clair and Ehrman 1996; Cirmo and McDonnell 1997), we found no seasonal pattern for TON-N retention efficiency.

Despite subsurface TN retention efficiencies being higher than surface retention efficiencies (García-García et al. 2009), the contribution of the subsurface component to whole wetland TN retention was very low (Table 4). This is a direct result of the low subsurface discharge. However, surface flow is usually absent in many slope wetlands and the subsurface component is the main pathway for the agricultural-derived water transport.

5 Conclusions

In conclusion, the study on slope wetlands shows high subsurface retention efficiencies for NO_3^--N (the dominant N form), with average annual NO_3^--N retentions in the same range as those described in riparian wetlands. High water temperatures, and indirectly we suggest prolonged water residence times, have positive effects on the efficiency of subsurface NO_3^--N retention. The studied wetlands show no evidence of saturation for subsurface NO_3^--N retention efficiency at high NO_3^--N concentrations, suggesting that these small wetlands may be considered suitable natural tools to control non-point source pollution in agricultural areas. Despite the low contribution of the subsurface component to total retention, the whole wetland N retention is high. By considering (1) the large number of wetlands which are usually located in the same agricultural catchment (as they are associated with the stream drainage network), and

(2) the wide distribution and strategic location of these Mediterranean stream wetlands at the landscape scale, our results clearly demonstrate their natural potential for controlling water quality, especially in agricultural catchments. Although our results were obtained from only two wetlands, they may contribute to develop N retention models in catchments where N export can be predicted and to establish the extent to which slope wetlands function as buffer systems. Nevertheless, further work on N retention processes would be helpful to incorporate good management practices in order to preserve them, especially when preservation may focus on the wetland complex rather than on a particular wetland. This is especially important if we wish to maximise the ecosystems’ N retention efficiency at the catchment scale. Preservation and/or restoration of semiarid Mediterranean aquatic ecosystems should take into account the potential use of these threatened wetlands complexes to enhance water quality.

Table 4. Total budget of the TN retention measured during the study period on the surface (see García-García et al. 2009) and subsurface components of the study wetlands. Values are mean ± SD (n=11 and n=12 in the Taray and Parra wetlands, respectively). In parentheses, we show the contribution to the total N inflow of the surface and subsurface components.

	Whole wetland			Surface component			Subsurface component		
	TN inflow (mg m^{-2} day^{-1})	TN net retention (mg m^{-2} day^{-1})	Total retention (%)	TN inflow (mg m^{-2} day^{-1})	TN net retention (mg m^{-2} day^{-1})	Contribution to total retention (%)	TN inflow (mg m^{-2} day^{-1})	TN net retention (mg m^{-2} day^{-1})	Contribution to total retention (%)
Taray	427.54 ± 383.40	355.50 ± 309.28	83.06	427.51 ± 383.40 (99.88 %)	355.47 ± 309.29	83.05	0.03 ± 0.02 (0.01%)	0.028 ± 0.02	0.01
Parra	307.66 ± 251.90	120.81 ± 81.51	39.23	307.47 ± 251.90 (99.84 %)	120.66 ± 81.52	39.18	0.19 ± 0.09 (0.07 %)	0.15 ± 0.08	0.05

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Chapter 3

Spatial variability in N and P retention through reaches in two Mediterranean slope wetlands

V García-García and R Gómez

Department of Ecology and Hydrology, Faculty of Biology, University of Murcia,
Campus of Espinardo, 30100 Murcia, Spain

Submitted to *Ecological Engineering*

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Abstract

It is well know that wetlands can significantly decrease the nitrogen (N) and phosphorus (P) concentration in agricultural surface and subsurface runoff, improving the water quality of aquatic ecosystems located downstream wetland. Nevertheless, some questions remain about the buffer function of wetlands as: does nutrient retention occur evenly throughout the total wetland length? or are there particular wetland portions more efficient than others? In areas under heavy utilization pressure, wetland conservation conflicts with the land use. In this sense, an efficient integrated water quality management and land use planning, demands the selection of the most suitable wetland sites and subsequently the dimensioning of wetland as buffer systems at catchment scale. Thus, the main objective of this study was to analyze the spatial variation in N and P retention through reaches in two Mediterranean slope wetlands (Taray and Parra) from agricultural catchments. Results obtained from showed that at wetland scale TP, and SRP were retained in Taray but they were exported in Parra wetland. In any case, the areal mass retention rates of TP within Taray (0.03 g m^{-2} y^{-1}), was low compared to other small wetlands. However, when we look for spatial differences in N and P mass retention through reaches, we observed that retention rates (mg m^{-1} dia^{-1}) had a non-linear character. Thus, the first 100 m of wetlands, the upper reaches, were significantly more effective at removing surface and subsurface NO_3^- and TN mass, than the lower reaches (200 and 300 m). Although differences in P mass retention between reaches were not statistically significant, a trend to be retained along the first 100 m of the wetland surface, and exported in the lower reaches, was also

observed for both, TP and SRP. The higher N and P mass retention in the upper reach of wetlands could mainly be related to assimilation by *Phragmites australis* and incorporation into biomas, P removal through formation of Fe plaques at root surfaces and N denitrification. Finally, our results show that a slope-wetland 100 m long, could remove a mean amount of 162-255 kg NO_3^- y^{-1} and 174-282 kg TN y^{-1}. On the basis of magnitudes of N and P fluxes estimates in agricultural landscapes under temperate climates, the preservation of a 10% of agricultural area to the slope wetland conservation, could remove the N mass exported from both, extensively and intensively managed agricultural landscapes.

Introduction

It´s well know that wetlands are able to efficiently transform and retain nutrients. Indeed, it have been proved that wetlands can significantly decrease the nitrogen (N) and phosphorus (P) concentration in agricultural surface and subsurface runoff, improving the water quality of aquatic ecosystems located downstream wetland (Young et al. 1980; Lowrance et al. 1984; Peterjohn and Correll 1984; Pinay and Decamps 1988; Mitsch 1992; Simmons et al. 1992; Jordan et al. 1993; Daniels and Gilliam 1993; Vought et at. 1994; Hoffmann and Kjaergaard 2009). As a consequence, nowadays it is increasing the recognition of these natural ecosystems as essential management tools for reducing the impact of agricultural nonpoint source pollution at catchment scale (e.g., Ardón et al. 2010; Chen and Hong 2012; Fan et al. 2012; Kröger et al. 2013). Thus, there is a growing awareness that wetlands are more valuable economic resources when retained in their natural or semi-natural state (e.g., Turner 1991). For instance, wetland conservation, wetland restoration or wetland construction is frequently suggested as an effective measure to combat aquatic ecosystem eutrophication (Mitsch et al. 2001).

Nutrient retention efficiency in wetlands vary widely because it depends of several biogeochemical and physical processes which are secondly controlled by a set of factors operating at different spatial-temporal scales (Groffman et al. 1988). At landscape scale, it is thus necessary to take into account the drainage basin area, the landscape setting of the wetland, the land use, the climate and the geology. At wetland scale, it is important the wetland morphology (depth, length and width) (Howard-Williams 1985) and the occurrence of vegetation inside the wetland (Weisner et al. 1994; Tanner et al. 1995; Sousa et al. 2010; González-Alcaraz et al. 2012a). At processes scale, nutrient availability, temperature, pH, carbon-availability and redox-conditions are the most relevant factors (e.g., Aldous et al. 2005; Moreno-Mateos et al. 2008).

Nevertheless, some questions remain about the buffer function of wetlands. For example, which is the minimum wetland surface to remove N and P efficiently ? Does nutrient retention occur evenly throughout the total wetland length, or are there particular wetland portions more efficient than others? Although a direct consequence of this “*new*” appreciation of wetlands services to humans it should be the implementation of sustainable management strategies for wetland protection and restoration (Chen and Hong 2012), in areas under heavy utilization pressure (e.g., in agricultural catchments), the wetland conservation conflicts with the land use. In this sense, an efficient wetland conservation, recreation or creation, demands as a first step the selection of the most suitable sites and subsequently to dimensioning of wetland as buffer systems at catchment scale.

For this purpose, it is necessary to establish the extent to which different wetland types may show high N and P retention efficiencies in the surface and subsurface pathways. Intuitively, larger and wider buffer zones should remove more nutrients from the water. For instance, Mayer et al. (2007) reported that buffer width is an important factor in managing N at catchment scale, so buffer width is positively related to surface N retention efficiency. They showed that wide buffers (> 50 m) more consistently removed NO_3^--N entering a riparian zone than narrow buffers (0-25 m). But, is this nutrient retention ability uniform through the wetland? Some authors highlight that nutrient retention in buffer zones has a non-linear character (e.g., Moreno et al. 2007). Thus, in the first part of the buffer (0-5 m from the upland-wetland edge) significantly more nutrients (20-60%) are retained than in the remote parts of the buffering ecosystems (e.g., Vought et al. 1994). Flite et al. (2001) also reported that the highest groundwater NO_3^- retention in an Appalachian Valley riparian system occurred closest to the source of NO_3^- near the upland-riparian edge. These studies have been successful in elucidating the importance of the distance from the upland-wetland edge on nutrient retention, but they have been conducted generally over short spatial scales (0-50 m) and at a relative narrow climatic range (template and continental zones).

In the sedimentary catchments of the Iberian Peninsula southeast, where the predominant land use is irrigated agriculture, slope wetlands are widely distributed (García-García et al. 2013). Previous studies in this type of wetlands showed annual average N retention efficiencies ranging between 50.9% and 87.9% for surface water (García-García et al. 2009) and between 82.2% and 91.3% for subsurface water (García-García et al. 2013).

However, as in other areas of the Mediterranean region, intensive agriculture supported by external water resources have resulted in many wetlands being disturbed or cleared out from the landscape through its desiccation. As a consequence, the reduction in the number of wetlands and the deterioration of aquatic ecosystems water quality have increased. Where wetlands have not been completely removed from the landscape, small (0.4-0.8 ha) and threatened wetlands appear spread out through the agricultural catchments. In these landscapes, where wetland conservation conflict directly with the human land uses, the wetland services recognition is the unique argument for ensure wetland preservation. For a suitable environmental planning at the catchment scale that ensure both, wetlands protection and water quality improvement, it is urgent to analyze the effect of the wetland spatial arrangement on the N and P retention efficiency. This is especially true in areas where human uses compete by space intensively.

The objectives of the present study were i) to analyze the surface and subsurface P retention efficiency along two slope wetlands draining agricultural catchments ii) to examine the spatial variability in surface and subsurface N and P retention through the different wetland reaches iii) to investigate factors controlling the spatial variability on N and P retention iv) provide guidelines for the preservation of wetlands in Mediterranean agricultural landscapes.

2 Materials and methods

2.1 Study site

The study was conducted in the Taray (0.5 ha) and Parra (0.7 ha) wetlands, two Mediterranean slope wetlands located within the catchments of the Salada and Parra streams, respectively (the Murcia Region, south east of Spain, 38º 9’ 26’’ N, 1º 9’ 4’’ W; 38º 13’ 93’’ N; 1º 4’ 23’’ W) (Fig. 1). In this part of the Mediterranean, average annual precipitation and temperature are close to 300 mm and 18 ºC, respectively (SIAM; Fortuna and Abanilla meteorological stations).

The dominant geology of the wetland catchments is sedimentary marl (from the Miocene) and the predominant land uses are the natural vegetation, constituted by Mediterranean shrubs, and the dry/irrigated crops. Further details about the geology and the land use are described in García-García et al. (2009). Wetland soils include

Hyposodic Hypogypsic Gypsisols and Hypergypsic Gleyc Solonchaks (FAO 1998), which have a silt loam to clay loam texture.

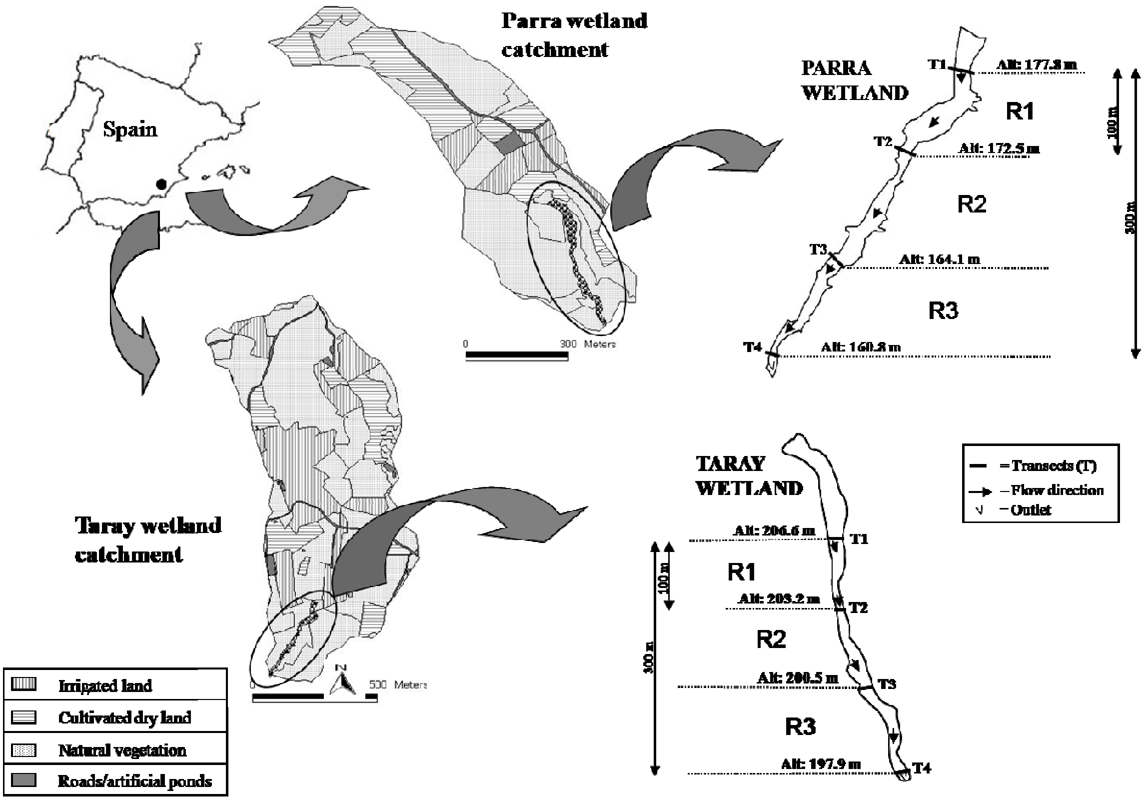

Fig. 1. Location and longitudinal view of the studied wetlands with the reaches (R1, R2 and R3). Alt indicates the altitude of each transect.

The study wetlands and the adjacent agricultural uplands are hydrologically connected via subsurface water and very occasionally, by overland water inputs generated by strong rainfalls. When subsurface agriculture-derived water intersects the soil surface in the upper part of the wetlands, it moves longitudinally (downslope) through the surface and subsurface components (see García-García et al. 2013 for slope wetlands description).

In both study wetlands, there was a clear pattern of vegetation distribution according to a water salinity gradient, which comprises the lowest values at the upland-wetland edge and the highest values at the wetland outlet. According to this gradient, the upper reach (R1) of both wetlands is almost completely cover by a well-developed reed population (*Phragmites australis*) follows by a halophytic shrub community (*Suaeda vera, Sarcocornia fruticosa, Arthrocnemun macrostachium*) combines with reeds (R2). Finally, the composition and the plant community cover in the last reach (R3) differ between wetlands. Whereas in the Parra wetland R3 is completely covered by a halophytic shrub community, in the Taray wetland the halophytic community is mixed with patches of *Juncus sp*., *Tamarix sp*. and bare soil. Along the three reaches, patches of *Vaucheria dichotoma* and a well developed periphyton community are present between the vegetation.

2.2 Field sampling

The study was conducted along the total length of wetlands (300 m) from the upland-wetland edge to the wetland outlet. Along the study length, four sampling transects (T1-T4) were established perpendicularly to the water flow direction at 100 m intervals, with the first transect (T1) located at the upland-wetland edge. Thus, three wetland reaches of 100 m-length (R1, R2 and R3) were limited by transects (Fig. 1).

Monthly water sampling was carried out from March 2007 to March 2008 in the Taray wetland, and from April 2007 to June 2008 in the Parra wetland (the Parra wetland was dry from July to September 2007). On each sampling date, surface and subsurface water samples were collected in the four transects (T1-T4).

Surface water samples were taken using plastic syringes (100 ml), as surface water depth ranged from 0.5 to 10 cm during the study period in both wetlands, and were stored in acid-washed polyethylene bottles under dark and cold conditions (4ºC) until to analyze them in the laboratory. The surface water samples collected by sampling date ranged from 7 to 11 in the Taray wetland, and from 10 to 13 in the Parra wetland (further details are described in García-García et al. 2009).

Subsurface water samples were collected from piezometers located at each of the four transects and installed perpendicular to the flow direction. From two to six piezometers were inserted by transect depending on wetland width, being located a total of fourteen and nineteen piezometers in the Taray and Parra wetland, respectively. On each transect, piezometers were spaced at 1 m intervals and inserted at a depth of 1 m below the sediment surface. They were PVC pipes (the internal diameter was 2.96 cm) perforated with 0.4 cm holes in the lower 70 cm. To avoid the ingress of rainwater and sediment inside the piezometer, the top and bottom were sealed with PCV caps. Subsurface water samples from piezometers were collected using a hand-operated peristaltic pump (Eijkelkamp Agrisearch Equipment B.V., Giesbeedk, the Netherlands).

Prior to piezometer sampling, the water table level was measured (Sounding apparatus, Eijkelkamp Agrisearch Equipment B.V., Giesbeek, the Netherlands). Positive values of the water table level refer to situations when it was above the ground surface, while negative values refer to situations when it was below the ground surface.

Prior to collecting the subsurface water samples, we evacuated three piezometer volumes of water from each piezometer. Subsurface water samples for the N and chloride (Cl^-) analyses were taken in acid-washed polyethylene bottles and were preserved under dark and cold conditions (4ºC) until to analyze them in the laboratory.

The water samples for the P analyses were collected in amber glass bottles and preserved with concentrated sulphuric acid to $pH < 2$. The temperature, salinity and conductivity of the surface and subsurface water samples were measured *in situ* using a conductivity meter (Tetracon 325; WTW, Munich, Germany).

2.3 Flow discharge calculations

On each sampling date, the surface flow discharge was only measured *in situ* on T4, so water sheet was so shallow in the rest of transects, and we based on the mean depth, total width and the mean current velocity measurements (current MiniAir2; Schiltknecht Co, Zürich, Switzerland). For estimating the surface flow discharge in the rest of transects, we considered Eq. 1:

$$D_{in} = (Cl^-_{out} / Cl^-_{in}) * D_{out} \qquad (1)$$

where D_{in} and D_{out} are the flow discharges ($l\ s^{-1}$) in the inlet and outlet of a reach respectively, and Cl^-_{out}/Cl^-_{in} is the concentration ratio of Cl^- in the outlet and inlet of the same reach. We had to start the discharge calculations from the outlet to the inlet of wetlands, following the next equations in that order:

1) $D_{T3} = (Cl^-_{T4} / Cl^-_{T3}) * D_{T4}$

2) $D_{T2} = (Cl^-_{T3} / Cl^-_{T2}) * D_{T3}$

3) $D_{T1} = (Cl^-_{T2} / Cl^-_{T1}) * D_{T2}$

As same as previous studies (Lowrance et al. 1984; Jordan et al. 1993; Sabater et al. 2003), the subsurface flow discharge of each reach was estimated based on the Darcy's equation (Eq.2):

$$Q = K\,I\,A \quad (2)$$

where Q is the flow discharge ($m^3\ h^{-1}$), K is the saturated hydraulic conductivity ($m\ h^{-1}$), I is the water table slope or hydraulic gradient ($m\ m^{-1}$), and A is the saturated area (m^2). The hydraulic conductivity of clay loam soils at saturation was estimated to be $3.6 \times 10^{-4}\ m\ h^{-1}$ (Klute and Dirksen 1986). The hydraulic gradient on each reach was calculated based on the distance and the difference of the mean water table level between consecutive transects (100 m). The saturated area was estimated by multiplying the saturated depth by a width of 1 m so that the unit loads could be compared among piezometers (Lowrance et al. 1984; Sabater et al. 2003). Saturated depth was estimated by assuming that each soil profile conducted water through the top 1 m.

2.4 Chemical analyses

Nitrogen and phosphorus dissolved forms were determined on filtered water samples (Whatman GF/F, 0.7 μm nominal pore size; Whatman International Ltd., Maidstone, England) within 24 hours of collection. Total nitrogen (TN) and total phosphorus (TP) were determined on unfiltered and frozen water samples. Nitrate (NO_3^-) was measured by a colorimetric method following cadmium reduction to nitrite-N (NO_2^-) (Wood et al. 1967). Nitrite-N was analysed by the diazotization method (Strickland and Parsons 1972). Nitrate-N was calculated by subtracting the NO_2^- obtained by diazotization. Ammonium-N (NH_4^+) was measured by the phenol-hypochlorite colorimetric method (Solorzano 1969). Dissolved inorganic nitrogen (DIN) was calculated as the sum of the NO_3^-, NO_2^- and NH_4^+. TN samples were digested with potassium persulphate (D'Elia et al. 1977) and the resulting NO_3^- was analysed by cadmium reduction using an automated ion analyzer (EasyChem Plus, Systea Analytical Technologies, Italy). Total organic nitrogen (TON) was estimated as the difference between TN and DIN.

Soluble reactive phosphate (SRP) was determined by the molybdenum-blue colorimetric method (Murphy and Riley 1962). TP was only determined on surface water samples. TP samples were digested with sulphuric acid and potassium persulphate (APHA 1985) and the resulting SRP was analysed as described previously. Particulate phosphorus (Part-P) was calculated as the difference between TP and SRP. Chloride concentration (Cl^-), used as a passive tracer, was analysed on filtered water samples (Whatman GF/F, 0.7 µm nominal pore size; Whatman International Ltd., Maidstone, England) within 48 hours of collection by the silver nitrate volumetric method (APHA 1985).

2.5 N and P retention calculations

The retention efficiency for the different N and P forms was calculated in the three reaches of both wetlands by considering Eq. (1) (Trudell et al. 1986):

$$\%R = (1 - (\text{nutrient}/Cl^-_{out} / \text{nutrient}/Cl^-_{in})) \times 100 \qquad (1)$$

Chloride was used in this equation as a passive tracer to correct the effect of a possible solute dilution (by surface or subsurface water inputs) or concentration (by evapotranspiration). The retention efficiency in R1 was calculated by considering, nutrient/Cl^-_{in} and nutrient/Cl^-_{out} as the concentration ratios of both solutes in the inlet (T1) and outlet (T2) of this reach, respectively. To calculate the retention efficiency in R2, we firstly calculated the retention efficiency from T1 (inlet) to T3 (outlet) and later, the retention efficiency in R1 was subtracted. The retention efficiency in R3 was calculated by measuring the retention efficiency between T1 (inlet) and T4 (outlet) and subtracting the retention efficiency measured between T1 and T3.

Nutrient net retention (mg h^{-1} m^{-1}) in each reach was calculated by applying the nutrient retention percentage (%) measured in such reach to the wetland inflow nutrient load (mg h^{-1}). The retained nutrient load could be slightly overestimated or underestimated by the use of Cl^- concentration values and a literature value for hydraulic conductivity to estimate the surface and subsurface discharge, respectively.

2.6 Statistical analyses

Diferences in N and P mas removal rates between reaches were tested using a mixed model with wetland reach and season as fixed factors, and wetland as random factor. To analyse the seasonal effect, months were grouped as follows: spring (March, April and May), summer (June, July, August and September), autumn (October and November) and winter (December, January and February). Simple linear regressions were used to calculate the best fitting regression model to explain N and P retention efficiency through reaches at the surface and subsurface wetland components. All analyses were done with SPSS (SPSS software version 15.0.1 for Windows; SPSS, Incorporated, Chicago, Illinois) and MLRs with done with R (R version 2.6.0 for Windows; R Development Core Team, Vienna, Austria).

3 Results

3.1Hydrological characterization of wetland reaches

In general, all hydrological variables experienced a gradual change through the wetland reaches (Table 1). The water table level progressively decreased over the reaches. It was generally above the sediment surface in the three reaches of the Taray wetland, but it was usually below the sediment surface in the last two reaches (R2 and R3) of the Parra wetland. The hydraulic gradient was very low in both wetlands, with maximum values observed in R1 and R2 in the Taray and Parra wetlands, respectively As a consequence of the low hydraulic gradient values, the subsurface discharge was also low in both wetlands but its pattern of spatial variation differed between wetlands In the Taray wetland, the subsurface discharge decreased progressively from R1 to R3. However, in the Parra wetland, the highest subsurface discharge was for R2 and the lowest for R1, according with the highest and lowest hydraulic gradient, respectively.

In the surface, wetland discharge was also low and slightly decreased throughout the wetland (Table 1).The seasonal average subsurface water temperature was above 15ºC in both wetlands and it increased as water table level decrease, that is, the lowest temperature was registered in R1 followed by R2 and R3. The subsurface water conductivity also increased from R1 to R3 in both wetlands (Table 1). At the surface water temperature was constant, around 14ºC, over the wetland reaches but water conductivity, increased from R1 to R3.

Table 1. Hydrological and physicochemical characterization and plant communities of wetland reaches (R1-R3) during the study period. Values are the mean ± SE (n=13 and n=12 for Taray and Parra wetlands, respectively). All reaches were 100 m long. Water table level positive values indicate water table elevation on the ground and negative values indicate water table depth under the ground.

TARAY	**R1**	**R2**	**R3**
Water table level (cm)	+5.73 ± 1.20	+1.67 ± 2.0	+1.46 ± 2.4
Hydraulic gradient (m m^{-1})	$6.10^{-4} \pm 1.10^{-4}$	$3.10^{-4} \pm 1.10^{-4}$	$1.10^{-4} \pm 2.10^{-5}$
Subsurface discharge (l m^{-2} h^{-1})	$19.10^{-5} \pm 4.10^{-5}$	$9.10^{-5} \pm 6.10^{-5}$	$3.10^{-5} \pm 1.10^{-5}$
Subsurface water temperature (ºC)	16.7 ± 1.3	16.9 ± 1.3	17.8 ± 1.4
Subsurface water conductivity (mS cm^{-1})	20.9 ± 0.5	25.5 ± 0.6	32.5 ± 1.2
Surface discharge (l s^{-1})	0.76 ± 0.2	0.74 ± 0.18	0.61 ± 0.15
Surface water temperature (ºC)	15.20 ± 1.4	15.01 ± 1.5	15.4 ± 1.5
Surface water conductivity (mS cm^{-1})	19.1 ± 0.4	22.13 ± 0.5	28.4 ± 1.96
Plant community	Emergent (100%) *P. australis*	Emergent (100%) Halophytic shrubs *P. australis*	Emergent (83%) Halophytic shrubs *Juncus sp.* *Tamarix sp.*
PARRA			
Water table level (cm)	+6.91± 0.95	-3.38 ± 1.7	-14.32 ± 3.27
Hydraulic gradient (m m^{-1})	$1.10^{-4} \pm 3.10^{-5}$	$16.10^{-4} \pm 3.10^{-4}$	$4.10^{-4} \pm 2.10^{-4}$
Subsurface discharge (l m^{-2} h^{-1})	$3.10^{-5} \pm 1.10^{-5}$	$59.10^{-5} \pm 8.10^{-5}$	$18.10^{-5} \pm 6.10^{-5}$
Subsurface water temperature (ºC)	15.2 ± 0.6	16.3 ± 0.7	17.0 ± 0.9
Subsurface water conductivity (mS cm^{-1})	17.4 ± 0.7	19.3 ± 0.6	19.0 ± 0.6
Surface discharge (l s^{-1})	0.74 ± 0.14	0.72 ± 0.14	0.71 ± 0.14
Surface water temperature (ºC)	14.5 ± 0.7	14.3 ± 0.8	14.21 ± 0.9
Surface water conductivity (mS cm^{-1})	15.83 ± 0.6	16.3 ± 0.7	16.72 ± 0.7
Plant community	Emergent (100%); *P. australis*	Emergent (100%) Halophytic shrubs *P. australis*	Emergent (100%) Halophytic shrubs

3.2 P retention efficiency

Mean annual inflow P concentrations to wetlands were much higher in Taray than in Parra, with mean values for SRP of 0.021 ± 0.009 mg l^{-1} and 0.002 ± 0.000 mg l^{-1}, respectively for Taray and Parra wetlands. Inflow P concentrations in surface water were highly variable over time (Fig. 2) and were dominated by SRP (62.7 ± 6.3%) in Taray wetland, with particulate and organic forms (Part-P) being less than 38% of TP (37.3 ± 6.3%). However, in Parra wetland Part-P was the dominant P fraction (63.3 ± 7.4%) over the SRP concentrations (36.7 ± 7.4%).

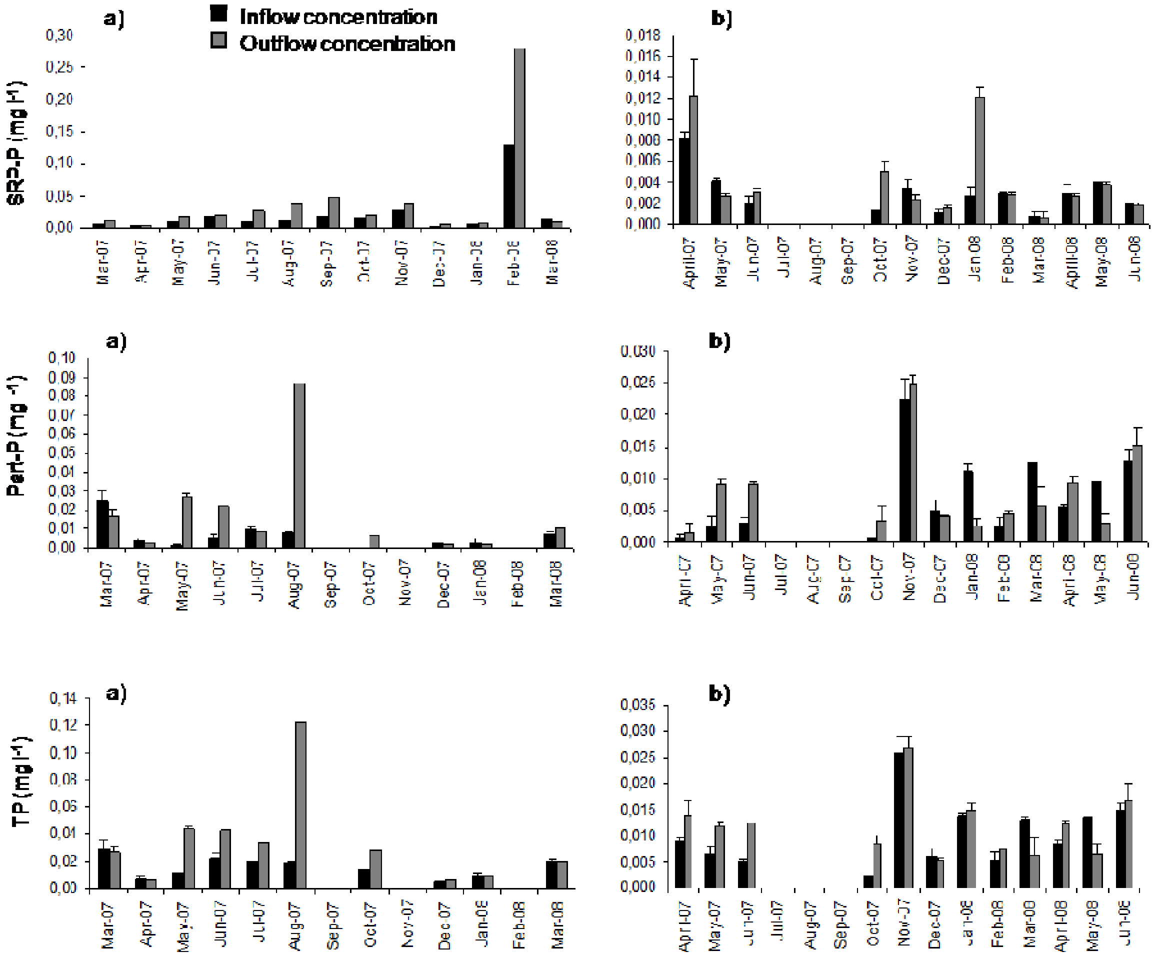

Fig. 2. Inflow and outflow mean SRP, Part-P and TP concentrations in the surface water of the Taray (a) and Parra (b) wetlands, over the study period. Values are the mean ± SE (n=10 and n=12 in the Taray and Parra wetlands, respectively.

At the entire wetland scale, surface P retention was very low. In fact, P retention values were negative in anyone of its forms during the most of the year. The mean annual SRP retention in Taray was the only positive retention value (10.83 ± 7.2%, against -39.91 ± 32.7% in Parra), for the rest of fractions all mean retention values were negatives. Part-P was the P form that showed the highest negative values and it was especially high in Taray (-343.0 ± 213%, regarding to -49.43 ± 31.4% in Parra). Mean annual TP retention values were -2.12 ± 19.9% in Taray and -31.1 ± 22.7% in Parra. TP outflow was again dominated by SRP in Taray wetland (57.0 ± 5.1%), although a slight increase in the percentage of Part-P was observed (43 ± 5.1%), regarding to the P inflow. In Parra wetland, similar percentages in SRP and Part-P were observed (37.7 ± 8.1% SRP and 62.3 ± 8.1% Part-P).

Subsurface SRP inflow concentrations were highly variable, higher than at the surface (Fig. 3), and similar between wetlands. However, P inflow load (μg $m^{-2}dia^{-1}$, Table 2) was much higher in Parra than in Taray wetland, contrary to the surface, where P inflow load was much higher in Taray than in Parra. SRP retention values differed between wetlands. Whereas Taray wetland showed a noticeable mean annual retention of 46.3 ± 7.5%, a negative mean retention value was registered in Parra, -269.2 ± 138.2%, negative values of SRP retention were especially registered during 2008. In any case, at surface or subsurface wetland a seasonal trend in P retention was not observed.

To calculate the areal mass retention of P in both wetlands, we applied the retention efficiency values to the inflow load on a monthly basis (Table 2). At the surface, Taray wetland showed a higher P mass retention than Parra, even when Taray received a higher P mass inflow. Relative to the mass inflow, Taray retained 13.4% of SRP, 41.3% of Part-P and 33.3% of TP. Whereas Parra exported, relative to the P mass inflow, 15.5% of SRP, 1.64% of Part-P and 2.15% of TP. At the subsurface, both wetland retained SRP, even considering that in Parra wetland SRP mass inflow was higher (5.5x) than at the surface. Relative to the mass inflow Taray wetland retained 60% of SRP regarding to 1.7% in Parra.

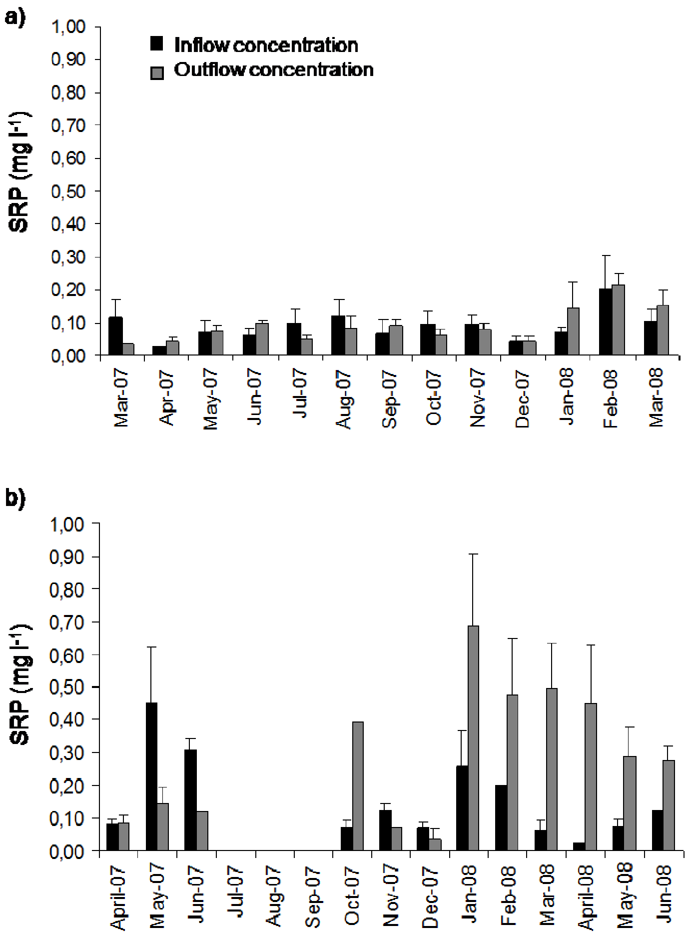

Fig. 3. Inflow and outflow mean SRP concentrations in the subsurface water of the Taray (a) and parra (b) wetlands, over the study period. Values are the mean ± SE (n=10 and n=12 in the Taray and Parra wetlands, respectively).

Table 2. Surface and subsurface inflow, outflow and net removal loads for P forms (mg m^{-2} d^{-1} for surface and µ m^{-2} d^{-1} for subsurface) in the studied wetlands . Values are the mean ± SE (n=13 and n=12 in the Taray and Parra wetlands, respectively). * (n=10). Negative values for net removal indicate P export.

	Taray wetland			Parra wetland		
	Inflow	Outflow	Net removal	Inflow	Outflow	Net removal
Surface						
SRP	0.097 ± 0.02	0.08 ± 0.02	0.013 ± 0.01	0.033 ± 0.01	0.038 ± 0.015	-0.005 ± 0.006
Part-P*	0.15 ± 0.10*	0.08 ± 0.03*	0.062 ± 0.07*	0.061 ± 0.01	0.062 ± 0.02	-0.001 ± 0.014
TP*	0.24 ± 0.11*	0.16 ± 0.05*	0.080 ± 0.07*	0.093 ± 0.019	0.095 ± 0.020	-0.002 ± 0.013
Subsurface						
SRP	0.015 ± 0.004	0.007 ± 0.001	0.009 ± 0.003	0.180 ± 0.066	0.177 ± 0.030	0.003 ± 0.071

3.3 N and P retention through reaches and mass removal rates

Retention efficiencies of the different N forms varied spatially among reaches (Fig. 4). NO_3^- was the dominant N form in both wetland components, representing the 89.9% of the TN in the wetland surface, and the 83.3% in the subsurface. NO_3^- was the only nutrient which showed a consistent pattern of spatial variation across wetlands: a decrease of concentration from R1 to R3 in the surface and subsurface water (Table 3).

At the subsurface, NO_3^- retention efficiencies decreased over the wetland reaches more gradually than at the surface (Fig. 4). In Taray, NO_3^- retention efficiencies in R1 were similar between wetland components (45.8 ± 3.4% and 48.5 ± 5.2%, for surface and subsurface, respectively). However in Parra, subsurface retention in R1 (82.7 ± 7.2%) was 2.3-fold as high than at surface (36.31 ± 7.32%).

The surface NO_3^- mass removal per unit length of wetland (mg h^{-1} m^{-1}, Table 4) varied significantly among reaches and seasons (Table 5), being R1 the wetland reach where it was recorded the highest net load removed in both wetlands. Seasonal differences were registered in spring and summer, when the highest (405.26 mg h^{-1} m^{-1} and 341.45 mg h^{-1} m^{-1} for Taray and Parra respectively) and the lowest (39.98 mg h^{-1} m^{-1} and 44.74 mg h^{-1} m^{-1} for Taray and Parra respectively) mass removal values were observed, respectively. Between autumn and winter no significant differences were observed with values of NO_3^- mass removal of 362.85 mg h^{-1} m^{-1} and 271.83 mg h^{-1} m^{-1} for autumn and winter respectively, in Taray and, 151.32 mg h^{-1} m^{-1} and 180.18 mg h^{-1} m^{-1} for autumn and winter respectively, in Parra. In the subsurface component, differences among reaches and seasons were not statistically significant (Table 5).

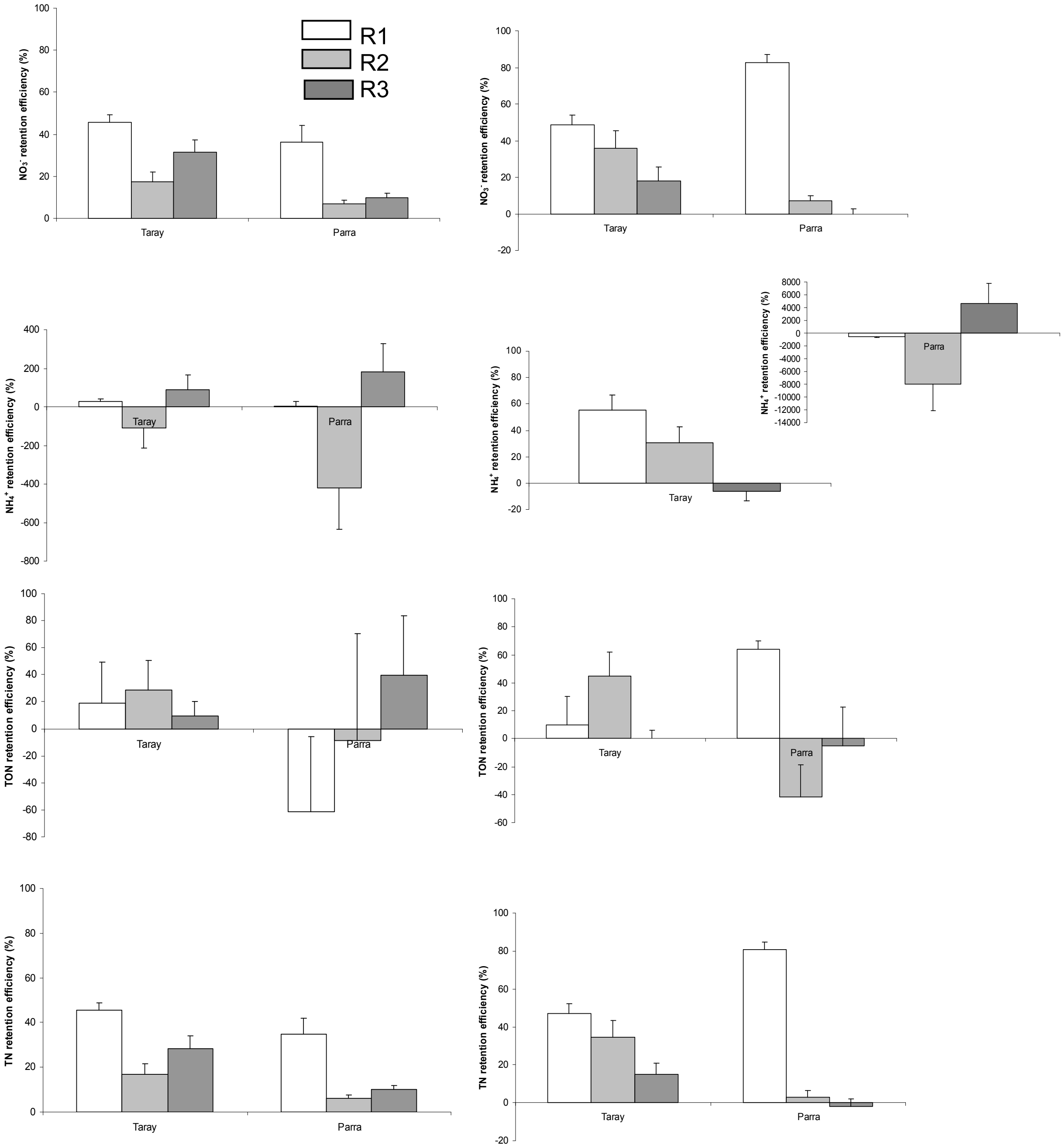

Fig. 4. Retention efficiency (%) of the different N forms through reaches in Taray and Parra wetlands. Values are the mean ± SE (n= 13 and n= 12 in the Taray and Parra wetlands, respectively).

Table 3. Concentrations (mg l^{-1}) of different N and P forms and Cl^-, measured at the wetland input and at the output of each wetland reach during the study period. Both, the surface and the subsurface wetland components are showed. Values are the mean ± SE (n=13 and n=12 for Taray and Parra wetlands, respectively and n=10 for TP, SRP and Part-P in Taray). Percentages of the different N and P forms respect to the TN and TP respectively, are also showed.

Surface component

	Taray				**Parra**			
	Input	**Output**			**Input**	**Output**		
		R1	**R2**	**R3**		**R1**	**R2**	**R3**
NO_3^-	21.5 ± 0.5	14.7 ± 1	12.7 ± 0.92	3.8 ± 0.75	27.4 ± 82.9	19.7 ± 3.1	17.7 ± 3	14.7 ± 2.6
NH_4^+	0.013 ± 0.003	0.010 ± 0.003	0.014 ± 0.003	0.020 ± 0.006	0.010 ± 0.002	0.009 ± 0.002	0.021 ± 0.005	0.016 ± 0.002
TON	2.4 ± 0.4	1.7 ± 0.3	1.8 ± 0.4	1.6 ± 0.3	2.5 ± 0.8	1.6 ± 0.5	2.1± 0.5	1.8 ± 0.6
TP	0.016 ± 0.002	0.016 ± 0.003	0.017 ± 0.003	0.034 ± 0.009	0.011 ± 0.002	0.008 ± 0.001	0.009 ± 0.002	0.012 ± 0.002
SRP	0.009±0.001	0.009±0.001	0.010±0.001	0.016±0.003	0.002 ± 0.000	0.002 ± 0.001	0.002 ± 0.001	0.004 ± 0.001
Part-P	0.006 ± 0.002	0.008 ± 0.003	0.008 ± 0.003	0.018 ± 0.025	0.009 ± 0.002	0.006 ± 0.001	0.007 ± 0.001	0.009 ± 0.002
Cl^-	3.2 ± 0.1	4.1 ± 0.1	4.6 ± 0.1	6.4 ± 0.4	3.7 ± 0.1	3.9 ± 0.2	4.0 ± 0.2	4.0 ± 0.2
%DIN	90.0	89.6	87.7	69.7	91.6	92.3	89.3	89.1
%TON	9.95	10.3	12.3	30.2	8.3	7.7	10.6	10.7
% SRP	59.3	53.1	56.5	46.4	21.3	25.7	23.7	30.3
%Part-P	40.7	46.9	43.4	53.6	78.7	74.3	77.6	69.6

Subsurface component

	Taray				**Parra**			
	Input	**Output**			**Input**	**Output**		
		R1	**R2**	**R3**		**R1**	**R2**	**R3**
NO_3^-	12.5 ± 0.6	8.3 ± 0.95	4.0 ± 1.0	0.8 ± 0.16	28.6 ± 3.4	8.2 ± 1.66	4.5 ± 1.36	3.66 ± 0.83
NH_4^+	0.47 ± 0.19	0.09 ± 0.02	0.07 ± 0.02	0.17 ± 0.05	0.01 ± 0.003	0.08 ± 0.02	0.86 ± 0.28	0.47 ± 0.13
TON	2.0 ± 0.2	1.7 ± 0.2	1.4 ± 0.08	1.7 ± 0.12	2.4 ± 0.53	1.0 ± 0.19	1.5 ± 0.32	1.6 ± 0.38
SRP	0.091 ± 0.012	0.146 ± 0.03	0.117 ± 0.019	0.091 ± 0.014	0.13 ± 0.02	0.15 ± 0.07	0.41 ± 0.09	0.33 ± 0.03
Cl^-	3.7 ± 0.05	4.4 ± 0.17	6.0 ± 0.21	7.7 ± 0.3	3.7 ± 0.2	4.8 ± 0.31	5.0 ± 0.2	5.0 ± 0.26
%DIN	85.6	82.6	74.2	35.8	92.0	88.8	75.6	71.5
%TON	13.3	17.1	25.5	64.1	7.8	11.0	21.0	27.4

Table 4. Annual inflow load to wetlands and mass retention rates of the different N and P forms in wetland reaches during the study period. Values are the mean ± SE (n=13 and n=12 in the Taray and Parra wetlands, respectively). *(n= 10). Negative values for net removal indicate export.

	Taray wetland				Parra wetland			
Surface water	Inflow load (mg h^{-1})	Net retention R1 (mg $m^{-1}h^{-1}$)	Net retention R2 (mg $m^{-1}h^{-1}$)	Net retention R3 (mg $m^{-1}h^{-1}$)	Inflow load (mg h^{-1})	Net retention R1 (mg $m^{-1}h^{-1}$)	Net retention R2 (mg $m^{-1}h^{-1}$)	Net retention R3 (mg $m^{-1}h^{-1}$)
TN	74299 ± 21802	285.3 ± 75.1	192 ± 71.7	200.4 ± 49.6	90178 ± 21442	234.8 ± 47.9	37.9 ± 8.4	80.2 ± 21.9
NO_3^-	65667 ± 18373	255.5 ± 65.0	170.3 ± 51.9	192.4 ± 46.3	84375 ± 19374	220.0 ± 41.0	41.2 ± 13.3	78.2 ± 26.4
NH_4^+	19.5 ± 4.5	0.06 ± 0.03	-0.06 ± 0.05	0.05 ± 0.04	41.5 ± 22.5	0.26 ± 0.25	-0.18 ± 0.06	-0.03 ± 0.05
TON	8610 ± 3741	26.7 ± 10.8	21.8 ± 18.3	7.7 ± 12.3	5727 ± 2467	14.1 ± 21.8	-2.9 ± 15.7	2.5 ± 13.1
TP *	40.3 ± 21.9	-0.03 ± 0.07	0.12 ± 0.11	0.09 ± 0.13	27.2 ± 5.5	0.09 ± 0.04	-0.01 ± 0.03	-0.07 ± 0.02
SRP*	19.9 ± 4.2	0.05 ± 0.01	0.00 ± 0.01	-0.02 ± 0.01	9.8 ± 3.3	0.001 ± 0.006	-0.01 ± 0.01	-0.01 ± 0.01
Part-P *	29.7 ± 20.1	-0.07 ± 0.08	0.13 ± 0.12	0.10 ± 0.14	17.8 ± 4.6	0.09 ± 0.04	-0.02 ± 0.02	-0.07 ± 0.03
Subsurface water	(µg h^{-1})	(µg $m^{-1}h^{-1}$)	(µg $m^{-1}h^{-1}$)	(µg $m^{-1}h^{-1}$)	(µg h-1)	(µg $m^{-1}h^{-1}$)	(µg $m^{-1}h^{-1}$)	(µg $m^{-1}h^{-1}$)
TN	490.9 ± 83.7	2.4 ± 0.6	1.1 ± 0.72	1.2 ± 0.8	7937.0 ± 1128.1	65.2 ± 10.8	3.4 ± 3.0	-3.2 ± 3.8
NO_3^-	413.7 ± 76.2	2.1 ± 0.5	0.8 ± 0.7	1.32 ± 0.9	726.4 ± 964.8	60.4 ± 9.6	5.4 ± 2.5	-0.42 ± 1.42
NH_4^+	27.8 ± 19.1	0.25 ± 0.19	0.015 ± 0.005	-0.007 ± 0.007	4.9 ± 1.4	-0.23 ± 0.09	-2.1 ± 0.9	0.84 ± 1.1
TON	71.6 ± 14.4	0.02 ± 0.16	0.33 ± 0.15	0.01 ± 0.04	707.1 ± 183.6	4.9 ± 1.4	-1.02 ± 0.9	-2.5 ± 3.04
SRP	3.12 ± 0.8	-0.01 ± 0.01	0.0.2 ± 0.01	0.01 ± 0.01	52.7 ± 19.4	0.30 ± 0.25	-0.5 ± 0.27	0.21 ± 0.23

In the wetlands, NH_4^+ concentrations were higher at subsurface (the 3.13 % of TN) than in the surface (0.05% of TN), especially for the case of Taray wetland. Through reaches, the NH_4^+ output concentrations did not show a gradual decrease but they varied considerably over reaches (Table 3). NH_4^+ retention efficiency was highly variable, through reaches and over the year. As a general trend it followed the same spatial pattern of variation in the surface component of both wetlands, with the highest annual retention efficiency observed in R3 (88.5 ± 71.7% and 180 ± 130% in Taray and Parra, respectively) and with negative values of retention in R2, that is, with R2 exporting NH_4^+ (Fig. 4). Values of NH_4^+ retention higher than 100% in R3, are not unexpected if we consider that R2 acted as a NH_4^+ source. In the surface, NH_4^+ net load removed per unit length (Table 4) showed significant differences among reaches (Table 5) with differences between R1 and R2 and between R2 and R3. On the contrary, NH_4^+-N net load removed did not differ significantly among reaches in the subsurface component. In any of the compartments seasonal differences in NH_4^+ net load removed were detected (Table 5).

TON concentrations in wetlands were higher than NH_4^+ and represented the 10% of the TN at the surface and the 13.3% at wetland subsurface. In general, TON input concentrations were diminished in both wetlands and in both components (Table 3), although the spatial variation of TON retention efficiency differed considerably between wetlands and components (Fig. 4). The TON net load removal did not differ significantly among reaches neither in the surface nor in the subsurface components. Differences between seasons were neither found (Table 5).

Table 5. Statistical results of the mixed-model analysis of variance to test for differences in the dependent variables: NO3, NH4, TON, TN, SRP, Part-P and TP removal rates mg h^{-1} m^{-1} between reaches (reach factor), among seasons (season factor), and the interaction between the 2 factors. ** $p < 0.0001$ $^{*}p < 0.01$ $^{+}$ $p < 0.05$

		Surface		Subsurface	
Dependent variable	Factor	F	p	F	p
NO_3^-	Reach	106.66	0.00^{**}	1.03	0.39
	Season	12.27	0.00^{*}	1.11	0.39
	Reach x Season	1.51	0.26	0.62	0.71
NH_4^+	Reach	6.62	0.013^{+}	0.81	0.47
	Season	0.04	0.99	0.18	0.91
	Reach x Season	1.40	0.30	1.00	0.47
TON	Reach	3.55	0.06	1.26	0.32
	Season	0.80	0.52	1.26	0.33
	Reach x Season	1.38	0.30	1.22	0.36
TN	Reach	255.61	0.00^{**}	1.06	0.38
	Season	9.83	0.00^{*}	1.74	0.22
	Reach x Season	1.26	0.35	0.76	0.62
SRP	Reach	3.31	0.07	1.34	0.30
	Season	2.97	0.08	0.21	0.89
	Reach x Season	0.27	0.94	1.41	0.29
Part-P	Reach	0.18	0.83	-	-
	Season	0.62	0.62	-	-
	Reach x Season	0.68	0.67	-	-
TP	Reach	0.57	0.58	-	-
	Season	0.61	0.62	-	-
	Reach x Season	0.79	0.59	-	-

Input concentrations of P to wetlands were very low, especially in the surface (Table 3). Over a year both, retention and export of small P loads from wetlands were observed (Table 4). However, the spatial variability in P retention through reaches was also highly variable and depended of the P form. TP was retained in the wetland surface (Fig. 5) at least over the R1 (22.02 ± 5.8% and 11.8 ± 22.5% in Taray and Parra, respectively), although on an annual basis, the high variability in wetlands retention efficiency explained why in Taray mean output concentrations from R1 and R2 were not different from the mean input concentrations (Table 3). R3 showed negative retentions in both wetlands (-11.6 ± 15.7% and -48.7 ± 16.1% in Taray and Parra, respectively, Fig. 5). Because the high variability of data, differences in the removal of

TP load between reaches were not statistically significant (Table 5). Differences among seasons were neither detected.

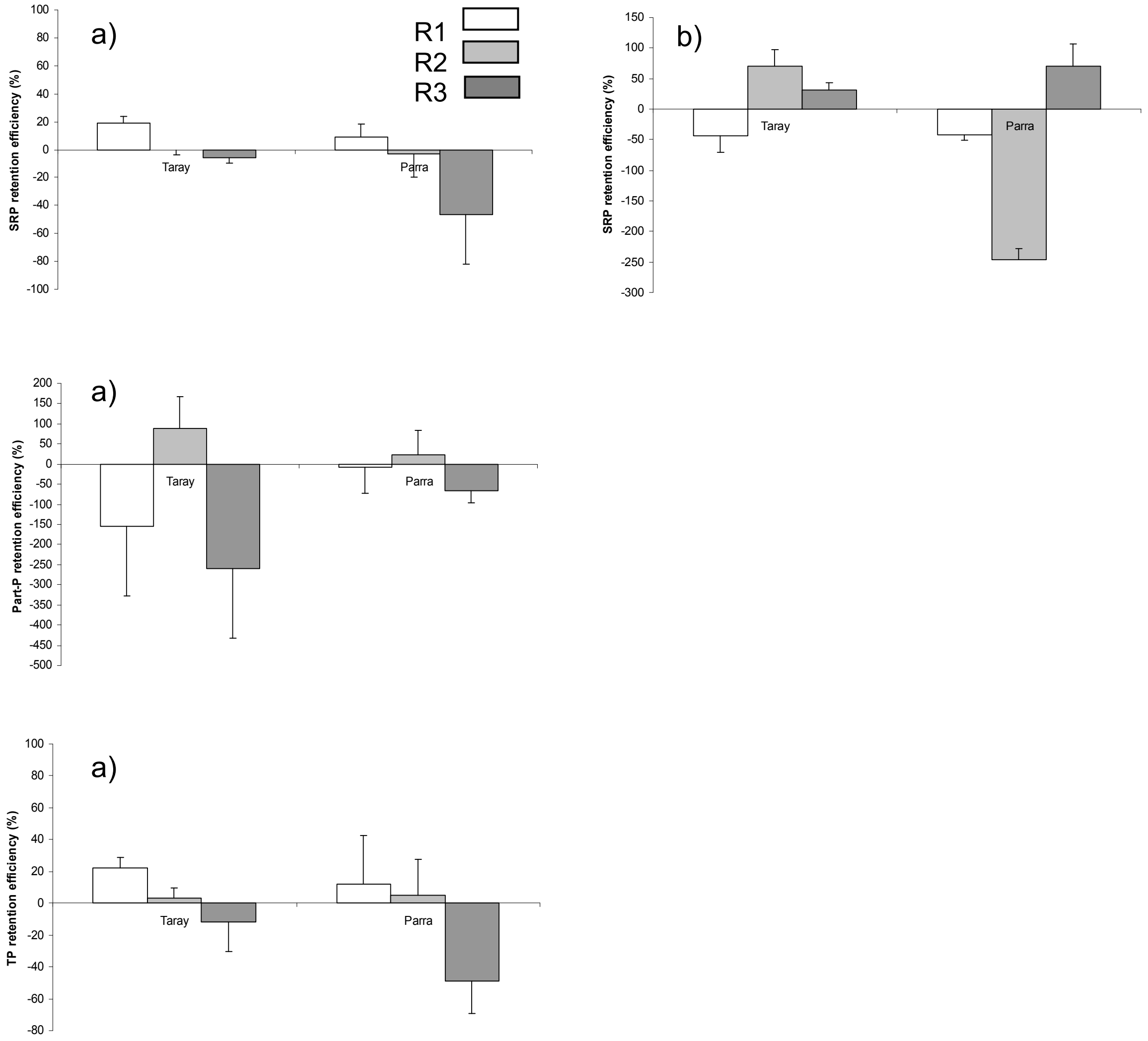

Fig. 5. Retention efficiency (%) of the different P forms through reaches in the surface (a) and subsurface (b) components of both studied wetlands. Values are the mean ± SE (n=10 and n=12 in the Taray and Parra wetlands, respectively).

Part-P was no retained through wetlands, on the contrary, it was usually exported form them. The spatial pattern of SRP retention, similar to that described for

TP, was consistent across wetlands (Fig. 5). Although a low retention was observed in R1 (19.15 ± 4.54% and 19.12 ± 8.19% in Taray and Parra, respectively), differences in the mean annual concentrations between the input and outputs from reaches were not observed (Table 3). Similarly to TP, R3 showed negative retentions for SRP in both wetlands (-15.73 ± 3.7% and -46.27 ± 31.50% in Taray and Parra, respectively, Fig. 5). At the subsurface, where SRP concentratiosn were higher than at the wetland surface, a slight increase in concentrations through reaches, respect to the wetland inputs, was observed (Table 3). SRP concentrations were more spatially variable in the subsurface water than in the surface water. In any case, differences in the removal of SRP load were not detected between reaches nor seasons in any wetland compartment (Table 5).

3.4 Driving factors on the N and P retention efficiencies over reaches

Results from the linear regression analyses carried out between the retention efficiencies for N and P through reaches and the environmental variables, differed between wetlands and wetland components (Fig. 6).

In Taray wetland, variability in the surface SRP retention through reaches was positively explained by differences in the water table level, and the NO_3^- and TN inflow concentrations to reaches. Similar results were obtained for the TP retention that was positively explained by the NO_3^- and TN inflow concentrations. However, the only significant correlation observed in Parra wetland was between the hydraulic gradient and the surface NH_4^+ retention efficiency. In this case, the NH_4^+ retention in reaches decreased as the hydraulic gradient increased. None other significant correlations were founded between nutrients retention efficiencies at the wetland surface and the studied environmental variables.

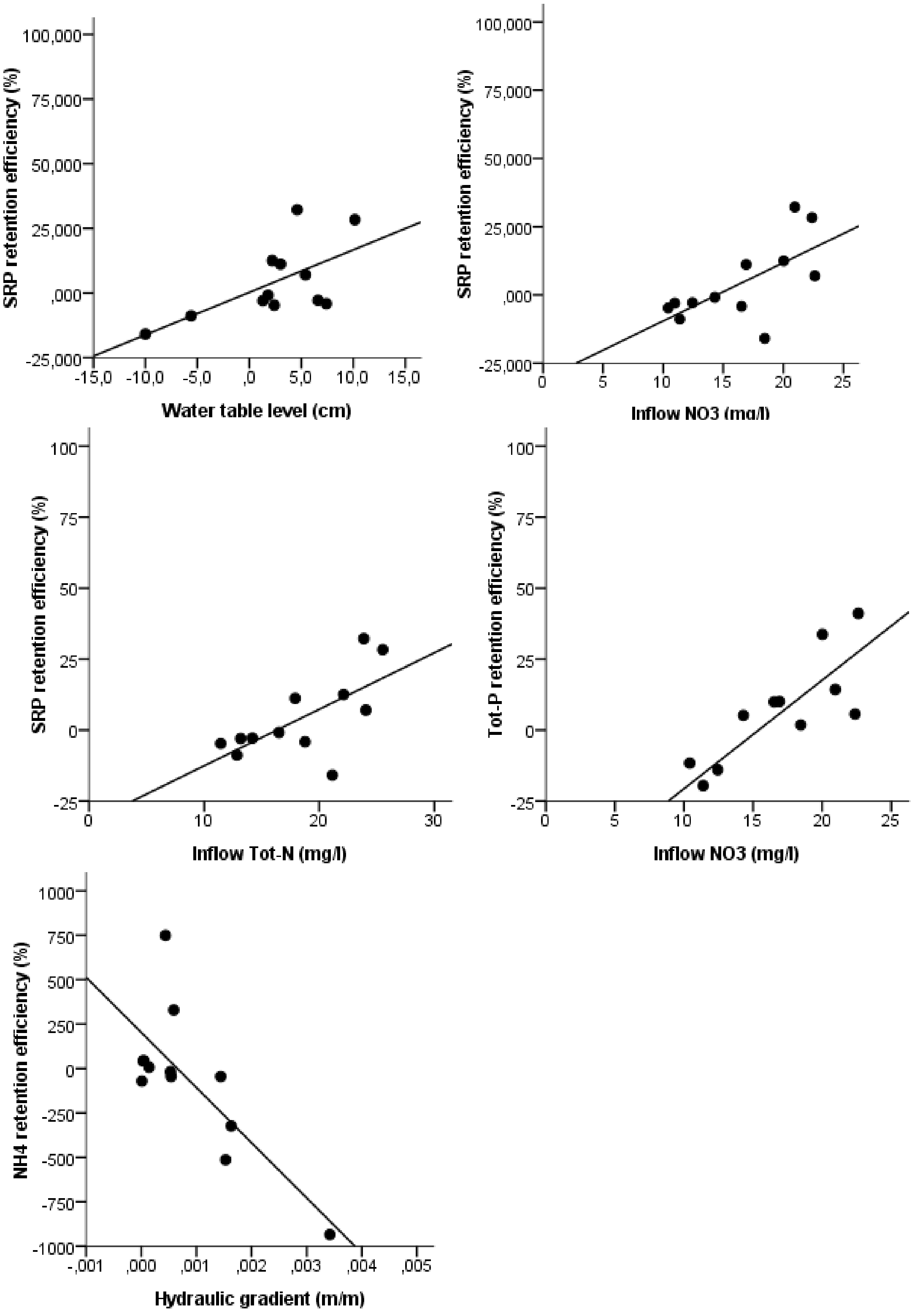

Fig. 6. Relationship between SRP, TP and $NH4^+$ retention and environmental variables (n=12).

At the subsurface, differences in N retention between reaches seemed to be explained by N concentrations in both wetlands. Thus, in Taray and Parra wetlands NO_3^- retention correlated positively with NO_3^- and TN inflow concentrations (whose dominant form was NO_3^-). Similarly NH_4^+ correlated positively in Taray with the TN inflow concentrations, and in Parra wetland the TON retention correlated positively with TN, NO_3^- and TON inflow concentrations to reaches. In Parra wetland variability in subsurface NO_3^- retention through reaches was also explained by differences in the water table level between reaches. None other variables were founded to explain other nutrient variation through reaches.

4 Discussion

4.1 P retention in the surface and subsurface components

Both wetlands received subsurface drainage water from upland agricultural field rich in N, mainly NO_3^-, and poor in P. This is probably due to both the clayey soil type which retains P well, and to the fact that the fields we have chosen were not fertilized with manure, which otherwise typically causes a net P surplus in some agricultural fields (Sims et al. 1998). In spite of the low inflow P concentrations, TP was retained in Taray but it was exported in Parra wetland. In any case, the areal mass retention rates of TP within Taray (0.03 g m^{-2} y^{-1}) was low compared to other small wetlands (restored or constructed) in agricultural areas, as it is showed by the review of literature values made by Ardón et al. (2010). Areal mass rates registered in Taray wetland were even lower than the mass rates reported by Kovacic et al. (2006) in small constructed agricultural wetlands even of smaller size (0.4 - 0.16 ha) whose rates were of 0.06 - 0.1 g m^{-2} y^{-1}.

Regardless, positive as much as negative values of P retention are usually reported in the literature. High efficiencies in TP retention has been usually attributed to occur when P is associated with surface runoff and sediment. Consequently, much of the TP settle out and is removed by sedimentation within wetland (Higging et al. 1993; Hey et al. 1994; Kovacic et al. 2006).

However, in the studied wetlands the majority of the TP entering the wetlands entered as subsurface runoff, the main pathway of water input. The fact that in Taray wetland SRP was the dominant form of TP might explain its higher mass removal rate (33% of the mass inflow) regarding to Parra wetland, where a 2% of the TP mass inflow was exported. In Parra wetland, the less available organic and particulate P was the dominant form of the TP mass inflow. Similarly, SRP was retained in Taray (13% of the inflow mass) but exported in Parra(15% of the inflow mass) at the wetland surface whereas both wetlands retained SRP at subsurface, with retention values especially high in Taray (60% and 1.7% of the inflow mass for Taray and Parra respectively). The lower P mass inflow, more stable environment and the high capacity of soils to adsorb P, might explain its higher retention in the subsurface component.

When considering the standing stock pools of P in soils and in the vegetation it is easy to understand that wetlands could potentially export SRP. SRP release from wetlands it is mainly attributed to decreases in sediment redox potential and subsequent release of Fe-bound P (e.g., Ardón et al. 2010; Hoffmann et al. 2012), which constitutes one of the major inorganic P pools in many soils (Boström et al. 1988; Reddy and De Laune 2008). Thus, fluctuations in the soil waterlogging conditions through wetland or over time can be enough to induce a release of SRP. The wetland drying out during summer months, as occurred in Parra wetland, and the consequent mineralization of organic P under aerobic conditions, may also favour the SRP release from soils (Aldous et al. 2005). In any case, the mean annual SRP mass exported from Parra wetland was as low as 1.8 mg m^{-2} y^{-1}.

Finally, when comparing through the literature P retention in wetlands, we must consider that noticeable differences exist between restored o created wetlands, and mature wetlands. While undisturbed wetlands often are net sinks for P (e.g., González-Alcaraz et al. 2012b), there is evidence that newly restoration of agricultural land that was previously wetland (an increasingly common practice in North America) initially release P to surface waters (e.g., Jordan et al. 2003; Aldous et al. 2005, 2007; Ardón et al. 2010), although it is likely to be a temporary process. Created wetlands however, tend to show greater efficiencies in P retention (63 - 96%) than in natural wetlands (4 - 10%), although may be comparable in terms of mass retention (Mitsch 1992). Regardless, have also been described that as wetlands age, the P budget tends to reach equilibrium, as P leaving (from release of decomposing plant material) is equal to the P entering (e.g., Howard-Williams 1985).

4.2 Spatial variation in N and P retention through wetlands

Heterogeneity in landscapes has taken a primary role in studies of large-scale biogeochemical fluxes, predominantly focusing on heterogeneous components within riparian catchments (Hedin et al. 1998; McClain et al. 2003; Gribsholt et al. 2007; Harms and Grimm 2008). Quantifying biogeochemical transformations in the landscape has long been a focus of wetland and coastal research, particularly regarding the quality of water delivered to downstream systems (Peterjohn and Correll 1984; Correll et al. 1992). Many such studies evaluate this role of a wetland as the difference between inputs and outputs of nutrients (Jaworski et al. 1992; Mortazavi et al. 2000; Sutula et al.

2001). Although these are important studies, they do not elucidate the sites of important biogeochemical transformations. More recently, this focus has broadened to include studies of the importance of various components, inherent to the ecosystem or landscape of interest, in a range of systems. These studies include those of geomorphic features (Hester and Doyle 2008), flow baffles (Ensign and Doyle 2005), upland-peatland ecotones (Mitchell and Branfireun 2005; Boomer and Bedford 2008), seepage banks (Duval and Hill 2007), points of flow path confluence (Hedin et al. 1998; Lewis et al. 2007; Dent et al. 2007), leaf litter (Sobczak et al. 2003; Ashkenas et al. 2004), and microbial biofilms (Mullholland et al. 2000; Mullholland 2004). The focus of these studies is where, and how, heterogeneity increases the reactive rate or surface area by which nutrient processing can occur.

As have been reported in previous studies (García-García et al. 2009; 2013) at the entire wetland scale, the masses of TN and $NO3^-$ were retained in the wetland surface, whereas those of $NH4^+$ and TON were mainly released. At the wetlands subsurface similar results were obtained with the exception than the TON mass was also retained. However, when we look for spatial differences in mass retention through reaches, we observed that N and P retention rates (mg $m^{-1}day^{-1}$) has a non-linear character, as previous studies have demonstrated (e.g., Vought et al. 1994; Moreno et al. 2007), that is, nutrient retention is not proportional to wetland flowpath length.

Among the different N forms, NO_3^- (the dominant N form) showed a clear spatial pattern of mass retention in both wetlands components. Thus, the first 100 m of wetlands (R1) were significantly more effective at removing surface and subsurface NO_3^- mass than the remote reaches (R2 and R3). In fact, the first 100 m of wetland removed 1.5x more mg NO_3^- $m^{-1}h^{-1}$ than R2 and 1.3x more than R3 in Taray, and 5.3x more and 2.8x more, respectively in Parra. The fact that none significant correlations were found with the hydrological and chemical variables analysed, together with the presence of a 100% cover of *Phragmites australis* in the upper reach, suggest that plant uptake could be partially responsible of the upper reach high efficiency, as uptake rates of *P. australis* have been reported as the highest between the rest of wetland plants (e.g., Gagnon et al. 2012). We also suggest that denitrification, although was not estimate in the studied wetlands, could highly contribute to the high NO_3^- removal from this reach. Higher NO_3^- concentrations and higher degree of soil waterlogging, and consequently low redox potentials, in the upper wetland reaches, would favour the NO_3^- removal by denitrification.

Denitrification has been usually reported to be the most important process in NO_3^- removal, especially in wetlands receiving NO_3^- as the dominant N form (Johnston 1991; Vymazal 2001; Trepel and Palmeri 2002). Although plant assimilation has been described as only accounts for a small percent of the total nitrogen removal in some constructed wetlands when the nitrogen load is high (Tanner et al. 1995), field studies in a salt marsh with similar climatic conditions, pointed out that both, plant uptake and denitrification were implicated in the removal of high amounts of inorganic nitrogen from agricultural activity (González-Alcaraz et al. 2012a).

Nitrate retention decreased to the lower reaches. Although the last two reaches showed similar mass removal rates, the retention efficiency in the last one (R3), with lower slope, tended to be higher than in R2 in both wetlands. The higher sediments waterlogging and more anoxic conditions, that were visible on the top of sediments in the last wetland reaches (R2), could explain these results. Besides, changes in morphology and vegetation through reaches may affect the internal flow patterns that can be of importance for how efficiently reaches remove N. Changes in flow patterns can result in much shorter nitrogen residences times, higher water velocities and less efficient wetland reaches (Howard-Williams 1985; Spieles and Mitsch 2000). NO_3^- retention efficiency decreased much more gradually through reaches in the subsurface component (Fig. 7), although higher differences were observed when mass retention rates (µg m^{-1} dia^{-1}) were calculated (Table 4).

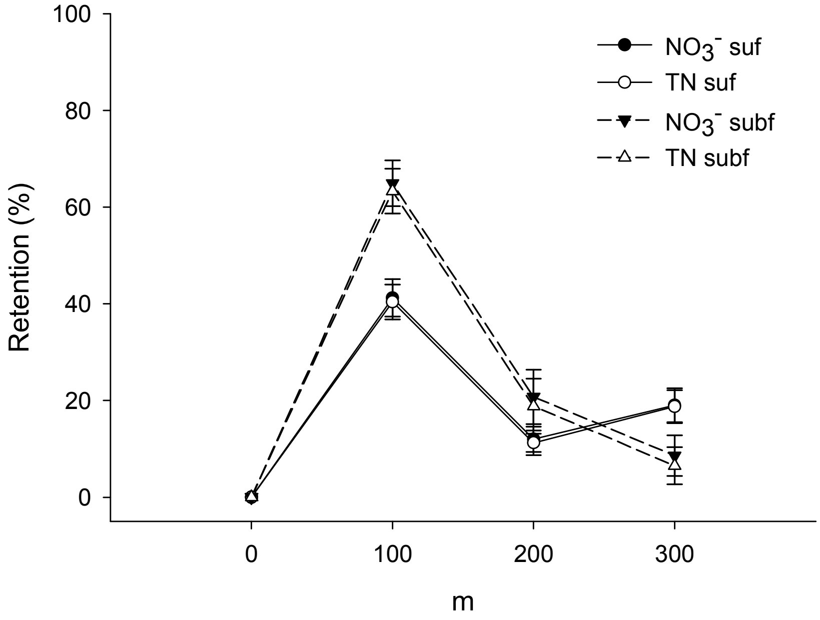

Figure 7. Retention percentages of TN and NO_3^- at surface and subsurface components related to the flow path length. Values are the mean ± SE (data from the two wetlands pooled, n=25)

In Taray wetland, a similar pattern in NO_3^- mass retention rates was observed trough reaches, with the upper reach removing 2.7x more µg NO_3^- m^{-1} day^{-1} than the middle reach (R2) and, 1.6 x more than the last one (R3), and this last one removing more µg NO_3^- m^{-1} day^{-1} than the middle reach. In Parra, this pattern was different, although the upper reach was the most efficient which removed in terms of mass 11.3x more NO_3^- than the rest of reaches. At subsurface component, variability in NO_3^- mass retention through reaches was related with N concentrations that support the idea of plant uptake and denitrification as main mechanism explaining NO_3^- retention, as both processes likely increase with N availability. As nitrification and mineralization (both processes increasing NO_3^- concentrations) are inhibited in the subsurface media, the effect of N inflow concentrations, flowing into reaches, on NO_3^- removal, cannot be masked as likely occur at the wetland surface.

Retention efficiencies of NH_4^+ and TON did not follow a spatial pattern, with the exception for NH_4^+ in the surface component where both wetlands showed the highest retention efficiency in the low reach (R3). We suggest than differences in retention efficiencies among reaches could be related to differences in NH_4^+ availability. Although in highly reducing environments, like those occurring in R3, nitrification was probably inhibited, and the NH_4^+ release from the organic matter/clay cation exchange complex would be favoured (Tiedje 1988; Silver et al. 2001), we suggest that NH_4^+ retention by microbial immobilisation could be stimulated by the higher NH_4^+ inflow load into this reach and lead a positive balance in NH_4^+ net retention.

Previous studies (Reddy and Patrick 1984; Hill and Warwick 1987) have already revealed that subsurface NH_4^+-N retention efficiency is stimulated by an increased inflow NH_4^+ concentration. The studied wetlands are NO_3^- rich systems and most organisms (bacteria, fungi and algae) prefer to assimilate NH_4^+ before NO_3^- as the first one it is energetically a less costly form of N (Stanley and Hobbie 1981; Patrick et al. 1985). The negative relationship founded between the hydraulic gradient and the NH_4^+ retention in Parra wetland support this hypothesis, as the increase of the hydraulic gradient reduce the water residence time in the reach and subsequently, the contact with the sediment biofilms and the assimilation rates, and its adsorption onto sediments. In fact, Gücker and Böechat (2004) underlined the importance of the water residence time on NH_4^+ retention efficiency.

Nevertheless, other previous studies have shown that NH_4^+ retention is stimulated by the hydraulic gradient (Cirmo and McDonnell 1997; Young and Briggs 2005). Regardless, NH_4^+ and TON are both N forms highly sensitive to changes of the local environmental conditions (e.g., discharge, water velocity, sediment redox potential, organic matter content) and this is consistent with the lack of an overall spatial pattern as was observed at the subsurface component.

Regarding P, overall the study wetlands were not effective removing P as they acted as P sinks at the entire wetland scale. However, although differences in P mass retention between reaches were not statistically significant, a trend to be retained along the first 100 m of the wetland surface, and exported in the lower reaches was observed (Fig. 5) for both, TP and SRP. The pattern of Part-P mass retention through reaches over the studied period was much more variable, although a slightly trend to be retained in the middle reaches was observed.

P retention in wetlands is extremely challenging due to the complex interactions between hydrology and biogeochemical transformations of P (Martin and Reddy 1997; Cirmo and McDonnell 1997). Overall, P retention is controlled by different physical, geochemical and biological processes although previous studies have emphasized either the role of abiotic processes (P sorption and precipitation) associated with fine sediments and the presence of iron (Fe) and aluminium (Al) oxides, and biotic uptake associated with plant and microorganism (Richardson 1985; Richardson and Marshall 1986; Reddy et al. 1995). Assuming that wetlands are heterogeneous ecosystems due to local environmental conditions (e.g., temperature, discharge, water table level, redox potential, vegetation), the dominant biotic or abiotic processes involved in P retention can vary spatially. Thus, were anaerobic conditions are present, Fe (III) oxides are subjected to microbially induced reductive dissolutions, which leads to partial of full dissolution of the Fe (III) oxides with a concurrent release of Fe (II) and release of adsorbed P to the solution (Pant and Reddy 2001; Patrick and Khalid 1974). Whereas under aerobic conditions, re-adsorption of dissolved P onto Fe (III) oxides occurs and precipitation of P takes place (Darke and Wallbridge 2000; Murray and Hesterberg, 2006).

Therefore, the decrease of SRP retention efficiency in the lower reaches, where sediments are dominated by silt and clay fractions and anaerobic conditions were present could be due to reduction of Fe oxides that bound P to soil particles (Richardson 1985). We suspect that, as occur in many other wetlands, interaction of P with the Fe and Al sediment complex in the lower wetland reaches was the ultimate responsible of P wetland release (e.g., Litaor et al. 2005). Whereas the higher P mass retention in the wetland upper reaches could mainly be related to plant uptake by *P. australis* and incorporation into biomas (Gagnon et al. 2012), has been also suggested for the case of NO_3^- mass retention. The fact that P retention at reach scale was explained by N availability, at least in Parra, support this idea. Besides, plants could also favour the P removal through root supply of oxygen or formation of Fe plaques at root surfaces (Davidson and Seed 1983; Christensen and Sand-Jensen 1998).

4.3 Evaluating water quality benefits

As have been described, agricultural runoff is mainly characterized by a high content of NO_3^-, whereas P tend to be in lower concentrations as P mass in the subsurface waters leaving agricultural soils tend to be retained by soils. In these slope wetlands the agricultural influenced water flows through the wetland mainly by the surface. Thus, wetland surface received the 99.9% and 99.7% of the N and P mass, respectively, coming from uphill sources.

To evaluate the capacity of the studies wetlands to control the non-point pollution in agricultural catchments and to give some utile data for water quality management and land use planning we use the study of Pärn et al. (2012) about the magnitudes of N and P fluxes in agricultural landscapes under temperate climate. In this study authors report, after an extensive literature review, that the overall N transport (that is N leaching and surface flow) from upland sources areas, ranged between the median amount of 15 - 75 kg N $ha^{-1}y^{-1}$ from intensive agricultural areas and of 1.5 - 19 kg N $ha^{-1}y^{-1}$ from extensively managed agricultural landscapes. Our results show that a slope wetland 100 m long can remove a mean amount of 162 - 255 kg NO_3^- y^{-1} and 174 - 282 kg TN y^{-1}. Or, considering a mean value of 10 m for the wetland width, that 0.1 ha of 100 m-long wetland is capable to remove such amount of N. In the Region of Murcia, as a mean value, the 53,5% of the land is agricultural land. The agricultural area is highly fragmented with the most of agricultural fields being less than 5 ha (LIC ES6200005- Humedal de Ajauque y Rambla Salada). That means, that a 100 m-long wetland could be enough to control de N fluxes from agricultural fields, in other words, with a 10% of agricultural area dedicated to wetland preservation, the N mass exported from both extensively and intensively managed agricultural landscapes, could be removed.

Our study highlight that the protection of at least, 100 m-long wetland, would be enough to improve the surface water quality in aquatic ecosystems, and point out the importance of their preservation or restoration in order to enhance the biodiversity at landscape scale. As vegetation, including halophytes (e.g., Sousa et al. 2010; González-Alcaraz et al. 2012a) are essential for nutrient removal in wetlands, wetland have to be preserved against human impacts that alter vegetation estructure and biomass through changes in the flooding regime and soil salinity (Álvarez-Rogel et al. 2007b). Wetland alteration threat two of the main important roles of these systems: the preservation of biodiversity and the buffering of agricultural runoff flowing into aquatic ecosystem down wetland. On the other hand, the ecosystem service that provides these small wetlands in relation to NO_3^- removal could be translatable to other similar landscape scenarios such as the ends zones of the reservoirs and therefore, their protection could also have a positive impact on the drinking water quality.

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General conclusions

General conclusions

1. The study wetlands showed high N removal efficiencies at both the surface (87.9% in Taray and 50.9% in Parra) and the subsurface wetland components (91.3% in Taray and 82.2% in Parra), even for high inflow N concentrations (27.2 - 27.5 mg l^{-1} and 17.7 -50.4 mg l^{-1} for Taray and Parra wetlands, respectively). Low discharge, high water temperatures and long water residence times in wetlands favored the biological processes implicated in N removal over the year. Thus, comparing with temperate wetlands, the warmer temperature in the Mediterranean wetlands was an adventage for N removal.

2. NO_3^- was the main N form in both wetland compartments. Besides, NO_3^- showed the highest retention percentages in both wetlands, with mean values of 72.3% at the surface and 93.2% at the wetland subsurface.

3. At the wetland surface, the hydrology and the NO_3^- inflow concentrations were the environmental factors affecting the NO_3^- removal. At the wetland subsurface, water temperature was the only environmental factor affecting NO_3^- retention.

4. In general, the highest NO_3^- retention efficiency was observed during the summer months, coinciding with the water temperature increase and the wetland discharge decrease.

5. In both wetland components, the NH_4^+ and TON retention efficiencies were much lower and more variable through the study period than the NO_3^- efficiency. In

some occasions, NH_4^+ and TON were exported from wetlands. Local variability (at microhabitat scale) in sediment redox and organic matter content could explain the N exportation.

6. Even though the subsurface wetland component showed a higher NO_3^- retention efficiency than the surface component, the lower subsurface discharge defined that the NO_3^- load removed at the subsurface (0.09 ± 0.08 mg m^{-2} day^{-1}, n=25) was much lower than the NO_3^- load removed at the surface (215.4 ± 217.7 mg m^{-2} day^{-1}, n=25).

7. In spite of the low inflow P concentrations to wetlands, at the entire wetland scale P was retained in Taray, in all its forms (SRP, Part-P and TP), but it was exported in Parra wetland. In any case, the areal mass retention rates of TP within Taray (0.03 g m^{-2} y^{-1}) was low compared to other small wetlands (restored or constructed) in agricultural areas.

8. The N and P retention efficiency in the study wetlands did not show a linear nature, that is, the increase in the wetland length did not cause a proportional increase in the nutrient retention.

9. The highest TN and NO_3^- (99.9% of TN) removal within wetlands was observed in the first 100 m (the upper wetland reaches). In these reaches, the mean NO_3^- retention at the surface was 41.2%, being of 64.9% at the subsurface in both wetlands.

10. In spite of differences between wetland reaches were not statistically significant, the NH_4^+ retention efficiency at the wetland surface was higher in the last wetland reach, where the high inflow NH_4^+ concentrations could stimulate the microbial

immobilization. TON retention efficiency did not show any consistent pattern of spatial variation, among reaches.

11. Althought differences in P mass retention between reaches were not statistically significant, a trend to be retained along the first 100 m of the wetland surface and exported in the lower reaches was observed for both TP and SRP. The sediment redox decrease in the lower reaches could explain the P concentration increase in the water column of this wetland area.

12. The presence and high biomass of *Phragmites australis* in the upper reaches of wetlands could be implicated in their higher N and P retention efficiency.

13. Our results show that a slope-wetland 100 m long, can remove a mean amount of 162-255 kg NO_3^- y^{-1} and 174-282 kg TN y^{-1}. Considering an overall N transport (that is N leaching and surface flow) from upland sources areas, ranging between 15-75 kg N $ha^{-1}y^{-1}$ from intensive agricultural areas and of 1.5-19 kg N $ha^{-1}y^{-1}$ from extensively managed agricultural landscapes, the use of a 10% of the agricultural area surface in the wetland preservation, could guarantee the N mass removal from the agricultural lands and the decrease of the P flux in a range from 11.8 ± 22.5% to 22.02 ± 5.8% .

14. The conservation and/or restoration of slope-wetlands could be an appropriate tool for the control of non-point pollution in agricultural watersheds. In this sense, the public recognition of their important functioning in agricultural landscapes, would be an useful strategy to guarantee their preservation. However we must not forget that the first step to improve water quality is to make lowering the use of fertilizers or to reduce the agricultural runoff. If we do not take this aspect into account, wetland could

be saturated with elevated nutrient mass inflows and consequently to alter wetland ecosystems, as same as other aquatic ecosystems downwetland.