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Nutrient export from largely pristine catchments (Serranía de Cuenca, Central Spain)

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ABSTRACT

We report here the summer nutrient (organic carbon, nitrogen and phosphorus) export from pristine catchments of the Serranía de Cuenca (Central Spain). These mountains are mostly encompassed by forested and rangeland landscapes and appear to have important groundwater outputs. On the basis of instantaneous sampling for nutrient chemistry, including in situ records of discharge in these largely non-gauged areas, nutrient fluxes downstream were estimated. Long-term (1982-2010) annual yields of total nitrogen and phosphorus downstream were also estimated from official data using discharge and concentrations at three larger sub-catchments, with the aim of relating them to the effects of global warming.
Average nutrient concentrations in these streams were rather low. The fraction of organic nitrogen content was relatively high, as in other forested catchments. Variability of concentrations changed from one catchment to another, but was wide. Organic carbon, total nitrogen and total phosphorus export downstream ranged from one- for organic carbon and nitrogen to three orders of magnitude for phosphorus, but these values lie within the lower quartile of reported export worldwide. There were no statistically significant relationships between discharge and nutrient concentrations. Water retention by lakes and reservoirs upstream decreased the variability of nutrients, particulate organic carbon and total nitrogen, but total phosphorus retention was unaffected. The catchment surface area and land use were unrelated with the phosphorus export. In-stream biological processes appeared to be negligible for nutrient export.
Contrary to the N export the annual P flux export was partly explained by discharge (and hence rainfall) in the long-term, which seemingly suggests a global warming effect for P.
Our results suggest that pristine catchments experience a complex behaviour of nutrient export that deserves further research, and should be more detailed to see if groundwater input plays a significant role.

Keywords: climate change; discharge; land use; nitrogen; organic carbon; phosphorus.

Exportación de nutrientes desde cuencas hidrográficas prístinas (Serranía de Cuenca, España)

RESUMEN

Estudiamos aquí la exportación estival de nutrientes (carbono orgánico, nitrógeno y fósforo) por las cuencas hidrográficas prístinas de la Serranía de Cuenca, en las cuales el agua subterránea puede jugar un papel importante, aunque poco conocido. Basándonos en muestreos puntuales de química de nutrientes y medidas in situ de caudales en esas cuencas no aforadas, estimamos el flujo de nutrientes aguas abajo de las mismas. También evaluamos la exportación anual a largo plazo (1982-2010) de nitrógeno y fósforo totales en las tres mayores subcuencas del territorio, usando datos oficiales e intentando relacionarla con los efectos del calentamiento global.
Las concentraciones promedio de los nutrientes en los ríos fueron bastante pequeñas. La de nitrógeno orgánico fue elevada, como en otros ambientes forestales. La variabilidad de las concentraciones cambió de unas cuencas a otras, pero resultó notable. La exportación de nutrientes varió de un orden de magnitud para carbono orgánico y nitrógeno a tres órdenes de magnitud para el fósforo, aunque las cifras se hallen en el cuartil inferior de la exportación por los cauces mundiales. No encontramos relaciones significativas entre el caudal y las concentraciones de nutrientes. La retención del agua en los lagos y embalses de la zona disminuyó las variabilidades de los nutrientes disueltos, del carbono orgánico particulado y del nitrógeno total, pero no la del fósforo total. Ni la superficie de las cuencas ni el uso del suelo pudieron relacionarse con las exportaciones de fósforo. La actividad biológica dentro de los cauces apenas afectó a la exportación de nutrientes.
A diferencia de la exportación anual de nitrógeno a largo plazo, la de fósforo quedó parcialmente explicada por el caudal (y la precipitación) en las tres grandes cuencas estudiadas, lo cual sugiere un efecto del calentamiento global para el P.
Nuestros resultados implican que las cuencas prístinas tienen un comportamiento complejo respecto a la exportación de nutrientes, el cual merece investigaciones futuras, que deberán ser más detalladas en ámbitos donde las aguas subterráneas jueguen un papel importante.

Palabras clave: cambio climático, carbono orgánico, caudal, exportación de nutrientes, fósforo, nitrógeno, uso del suelo.

VERSIÓN ABREVIADA EN CASTELLANO

Introducción y metodología

La cantidad de cuencas fundamentalmente prístinas está disminuyendo en todo el mundo. No hay demasiados trabajos sobre su exportación de nutrientes aguas abajo de las mismas y lo mismo puede aseverarse para la Península Ibérica. Por eso, hemos llevado a cabo un estudio en las subcuencas de la Serranía de Cuenca (Fig. 1, Tablas 1-2), que drenan hacia las del Alto Tajo y el Alto Júcar. Dichas subcuencas están tapizadas fundamentalmente por bosques y por tierras abandonadas, con muy poca población humana, por lo cual se hallan en muy buenas condiciones ambientales al apenas recibir aguas residuales urbanas. También tienen una agricultura cuya actividad ha disminuido notablemente en las últimas décadas. Además y aunque no es posible cuantificarlo aún, los caudales se ven incrementados por las descargas de agua subterránea.

Para este estudio, llevamos a cabo los muestreos en el verano de 2017 en 27 subcuencas de la Serranía, cuando determinamos las concentraciones y flujos de carbono orgánico, nitrógeno y fósforo, tanto en sus formas particuladas como en las disueltas. In situ medimos el caudal, la temperatura del agua, el oxígeno disuelto, el pH y la conductividad eléctrica en cada estación de muestreo. Usamos datos oficiales (Sistema de Información Geográfica de la Política Agraria Comunitaria, SIGPAC) para conocer distintas variables de las cuencas, tales como su superficie, su pendiente promedio y sus porcentajes de uso del suelo. Esas informaciones nos permitieron estimar los flujos instantáneos de nutrientes (totales y disueltos) debajo de cada subcuenca, los cuales –en conjunción con los datos ambientales– usamos para buscar factores de control de dichos flujos de exportación. También estimamos la exportación o retención netas por unidad de longitud fluvial para cada nutriente particulado, disuelto y total. Los resultados aportan la primera imagen puntual de la exportación de nutrientes en estas cuencas prístinas, útil a efectos comparativos con otras futuras, acusen el cambio global o no.

Además, analizamos la exportación anual aguas abajo de nitrógeno y fósforo totales a largo plazo (1982-2010) en tres grandes subcuencas (Cabriel, Guadiela y Escabas), basándonos en datos oficiales de las Confederaciones Hidrográficas de Tajo y Júcar y de la Agencia Estatal de Meteorología (AEMET). Nuestra intención era relacionar dicha exportación anual con algunas variables asociadas al cambio climático, tales como la pluviosidad, la temperatura del aire y sus variabilidades.

Los análisis estadísticos empleados para explorar las relaciones entre variables fueron tanto de carácter no paramétrico como un análisis de componentes principales (PCA).

Resultados y discusión

Las relaciones entre precipitación y caudal en el año anterior a nuestros muestreos difirieron entre las cuencas del Alto Júcar y el Alto Tajo, pues no resultaron significativas en el primer caso. En general, los caudales durante ese año fueron mucho menos variables que la precipitación (Fig. 2). Es muy probable que los aportes de aguas subterráneas a los caudales sean responsables de esas diferentes variabilidades y modifiquen las relaciones precipitación-caudal en estas cuencas kársticas.

Las concentraciones promedio de nutrientes sugieren que los cursos de agua de la Serranía de Cuenca se hallan muy limpios en su inmensa mayoría (Tabla 3). Tanto el nitrato como el carbono orgánico disuelto fueron las fracciones principales del nitrógeno total y del carbono orgánico, respectivamente, siendo el nitrógeno orgánico alrededor de un cuarto del N total (27 ± 21%) en todo el conjunto de ríos y arroyos. La variabilidad de nitrógeno y fósforo totales fue mayor en la cuenca del Alto Tajo, pero la del carbono orgánico disuelto resultó superior en la del Alto Júcar (Tabla 3). En general, la actividad biológica dentro de los cauces resultó despreciable para afectar a los procesos asociados a los nutrientes.

Los rangos de variación de carbono orgánico, nitrógeno y fósforo totales oscilaron entre 1,56-11,02 mg C/m^2/d, 0,30-5,71 mg N/m^2/d and 0-0,221 mg P/m^2/d, respectivamente (Tabla 4). Dado que solo pudimos hacer una estimación de caudal, la variabilidad de los flujos de exportación de nutrientes siguió estrechamente la de las concentraciones. La retención neta de nutrientes por unidad de longitud fluvial (es decir, el hecho de que hubiese menor flujo de nutrientes en los tramos más bajos que en los más altos de un mismo río) fue muy variable, tanto según los distintos nutrientes como entre las subcuencas (Tabla 5). A menudo, los flujos presentaron una retención neta, como ocurrió con el nitrato en los ríos Cabriel, Cuervo y Júcar o con el amonio en Cabriel, Cuervo, Escabas, Guadiela y Júcar (Tabla 5). Y el mismo río pudo experimentar retención neta para carbono *orgánico y nitrógeno y exportación neta para nitrato, amonio, nitrógeno total, ortofosfato y fósforo total, como sucedió en el río Mayor (Tabla 5).*

En contra de lo esperado, no constatamos una relación estadísticamente significativa entre el tamaño de las subcuencas y las concentraciones de nutrientes en sus aguas ($p > 0,10$). Los lugares más prístinos ($n = 7$) fueron menos variables para el carbono orgánico disuelto y el fósforo total que los demás (test de la U de Mann-Whitney, $p = 0,053$ and 0,077, respectivamente), pero el resto de los nutrientes no mostró diferencias entre sitios prístinos y otros algo más alterados ($p > 0,10$).

Para el total de los datos no encontramos relaciones estadísticamente significativas entre caudal y concentraciones de nutrientes ($p > 0,10$). Los pueblos próximos a cada subcuenca no afectaron aparentemente a dichas concentraciones, ya que su presencia ni las aumentó ni las disminuyó (test de la U de Mann-Whitney, $p > 0,10$). Sin embargo, la retención del agua por los lagos y embalses presentes en las subcuencas sí incidió sobre las concentraciones y flujos de NH_4, NO_3, nitrógeno total y carbono orgánico particulado y disuelto (test de la U de Mann-Whitney, $p = 0,052$-0,070), de modo que su variabilidad se veía reducida aguas abajo. Y lo mismo sucedió con los flujos de ortofosfato, aunque los de fósforo total no quedaron afectados ni por la retención del agua, ni por la presencia de pueblos aguas arriba ($p > 0,10$).

El tamaño de las cuencas tampoco se relacionó con ningún flujo de exportación de nutrientes ($p > 0,10$), en oposición a lo que sucede en otras cuencas del mundo. Solo el de aquellas subcuencas dominadas por tierras agrícolas mostró una correlación con el flujo de nitrato ($R^2 = 0,47$; $p = 0,006$), pero los demás flujos de exportación no se vieron controlados ni por el tamaño de cada cuenca individual, ni por el uso del suelo en ella. Curiosamente, tampoco las áreas forestales se relacionaron con flujo alguno de nutrientes ($p > 0,10$). El flujo de carbono orgánico total dependió del de carbono orgánico disuelto ($R^2 = 0,98$, $p = 0,00005$), mientras que el del nitrato se relacionó inversamente con el de nitrógeno orgánico ($R^2 = 0,55$, $p = 0,003$), algo típico en ambientes predominantemente forestales, como son algunos de los estudiados.

A fin de establecer mejor el conjunto de variables de control sobre la exportación de los nutrientes, realizamos varios análisis de componentes principales con los datos estivales de exportación y los datos de las variables ambientales. Los tres primeros factores del análisis de componentes principales sobre el conjunto total de los datos de exportación de nutrientes explicaron el 59,4% de la varianza total, cuyo primer Factor explicó más del 30% de aquella (Tabla 6). Los flujos de exportación de nutrientes se relacionaron con el Factor I, el cual se veía representado por el uso del suelo (% de tierras agrarias, tierras abandonadas y bosques) y la salinidad del agua fluvial. En cuanto al Factor II, venía explicado por el tamaño de las subcuencas, su pendiente promedio y el caudal. El factor III lo fue por el uso del suelo, aunque con mayores correlaciones que el Factor I (Tabla 7).

Cuando consideramos las cuencas del Alto Júcar y el Alto Tajo por separado, el caudal (Q) se relacionó con las concentraciones de amonio y fósforo total en la primera ($R^2 = 0,37$ y 0,56, $p = 0,026$ y 0,003, respectivamente) y –en cambio– lo hizo con la de nitrato en la del Tajo ($R^2 = 0,30$, $p = 0,041$). Las funciones potenciales (es decir, $Y = a*X^b$) dependientes del caudal, sin embargo, solo fueron significativas para el amonio en la cuenca del Alto Tajo ($[N\text{-}NH_4] = 0,015*Q\text{-}0,31$; $R^2 = 0,24$, $p = 0,08$). O sea, las cuencas se comportaron de manera quimiostática para la mayor parte de los flujos de exportación nutritiva, lo cual significa que las concentraciones de nutrientes no dependieron del caudal. El tamaño de la cuenca no controló ninguna concentración en el Alto Júcar, pero la del nitrato parece que dependió de dicha área en el Alto Tajo ($R^2 = 0,59$, $p = 0,001$). La extensión de los bosques tampoco se relacionó con ningún flujo de exportación de nutrientes en los medios fluviales que los drenaron ($p > 0,10$).

Una vez separadas las subcuencas del Alto Júcar y Alto Tajo, los tres primeros factores del análisis de componentes principales explicaron un 65,6% y un 77,5% de la varianza total, respectivamente. En ambos análisis, el primer Factor explicó más del 30% de la variabilidad total (Tabla 6) y la mayor parte de los flujos de exportación nutritiva se relacionaron con el Factor I en la cuenca del Júcar, con el cual se vinculaba la salinidad del agua fluvial. El Factor II quedó explicado por el caudal y la pendiente promedio de las subcuencas, al cual se asociaron los flujos de amonio y de fósforo total, mientras que el Factor III –ya sin relación con ningún flujo de exportación– podría considerarse como una manifestación del uso del suelo. En las cuencas del Alto Tajo, la mayor parte de los flujos se vincularon al Factor I, explicado mayormente por el uso del suelo (cultivos, tierras abandonadas, bosques); el Factor II se asoció levemente al flujo de amonio y quedó explicado por la pendiente promedio, el caudal y la conductividad del agua, mientras que el Factor III pudo representar el ambiente abiótico propiamente fluvial (temperatura del agua, oxígeno disuelto y pH; Tabla 7).

A largo plazo, la precipitación mensual explicó escasa, aunque significativamente, el caudal mensual con un mes de desfase en las tres grandes subcuencas analizadas (1982-2014; $R^2 = 0,24$, 0,26 y 0,27, $n > 400$, $p < 0.05$, *para Cabriel, Escabas y Guadiela, respectivamente). Es muy verosímil que los aportes de aguas subterráneas expliquen mejor la variabilidad de los caudales, pero no existen datos al respecto.*

*La exportación anual de nitrógeno por las grandes subcuencas del Alto Tajo (Guadiela y Escabas) mostró una pauta bimodal a largo plazo; sin embargo, la del fósforo resultó muy fluctuante (Figs. 3-4). En la cuenca del Alto Júcar, la exportación por el Cabriel presentó varios máximos para ambos nutrientes (Fig. 5). No hubo relación entre nutrientes totales o disueltos y el caudal para el conjunto de los datos de las cuencas de Guadiela y Cabriel (n > 100, p > 0,10), pero el nitrógeno total sí se relacionó significativamente con el caudal (Q) en el Escabas ([TN] = 5,22*Q-0,69; R^2 = 0,24, n = 105, p = 0,0003). Como no hay datos oficiales a largo plazo sobre carbono orgánico en estas cuencas, no pudimos llevar a cabo análisis parecidos con dicha sustancia.*

La precipitación anual controló parcialmente los flujos de exportación de fósforo en las tres grandes subcuencas estudiadas (R^2 = 0,16, 0,17 y 0,42 para Guadiela, Escabas y Cabriel, respectivamente; p = 0,012-0,0003), gracias a la influencia del caudal anual, el cual dependió de la precipitación y de su variabilidad (R^2 = 0,12-0,48; p = 0,005-0,0002). Cabe destacar aquí, de paso, que el caudal anual de las tres subcuencas se correlacionó significativamente (R^2 = 0,48-0,70; p = 0,0004-0,00006), a pesar de que la del Guadiela está regulada y las otras dos no. Este hecho sugiere que las tres subcuencas podrían funcionar de una manera hidrológicamente similar. De todos modos, la importante aportación de las aguas subterráneas en estas cuencas kársticas a la escorrentía superficial afectaría de modo notable las relaciones entre pluviosidad y caudales, pero no ha podido cuantificarse al tratarse de un territorio poco conocido hidrogeológicamente.

El promedio anual de la temperatura del aire y su variabilidad, en cambio, no mostraron relación alguna con la exportación de nutrientes en estas subcuencas (p > 0,10).

Por lo tanto, parece probable que el cambio climático relacionado con la pluviosidad haya tenido ciertos efectos sobre la exportación anual de fósforo a largo plazo en la Serranía de Cuenca, si bien sus efectos sobre la de nitrógeno parecen despreciables. Sin embargo, no se ha constatado que el efecto térmico del cambio climático tenga influencia en la exportación anual de nitrógeno y fósforo a largo plazo por las cuencas en los cursos de agua de la Serranía de Cuenca. Cabe la posibilidad, no obstante, de que la longitud de la serie de datos (1982-2010) aún no sea suficiente para detectar esa posible influencia.

En conjunto, nuestros resultados sugieren que las cuencas prístinas muestran un comportamiento complejo en cuanto a la exportación de nutrientes, el cual merece ulteriores estudios. Su análisis pormenorizado permitiría separar los efectos puramente naturales de los debidos al cambio climático, los cuales resultan más difíciles de detectar en cuencas donde otros impactos humanos enmascaren aquellos. De todos modos, la situación se hará más compleja en cuencas, como las kársticas, puesto que allí los verosímiles aportes de aguas subterráneas modificarán mucho las relaciones entre caudal y exportación de nutrientes. Esta clase de estudios está aún en sus comienzos.

Introduction

Since earlier attempts by Likens *et al.* (1970), nutrient export from streams has been a favourite topic for ecologists. It has often been considered (Likens and Bormann, 1974; Hynes, 1975) that such an export is a signature of catchments and reflects many ecological processes (erosion, leakage, nutrient processes, land use change and so on) which may occur there.

Early export studies have attempted to show the dependence of nutrient export on watershed size and water availability due to rainfall, runoff and discharge (Likens *et al.*, 1970). Later, other catchment features have been taken into account, such as human population, areas of distinct land use (*i.e.* forestry, agriculture, cattle feedlots, urban, rangeland...; Johnson *et al.*, 1997), often arriving at the obvious conclusions that firstly the dominant land use controls nutrient export, and secondly pristine catchment export lower fluxes than either urban- or crop-dominated catchments (Caporali *et al.*, 1981; Cooke and Prepas, 1998). Nevertheless, nutrient export downstream is the outcome of river import from both stream, groundwater (sometimes) and the terrestrial environments around a given reach (or catchment; Bencala, 1993; Johnson and Gage, 1997), as well as in-stream biogeochemical processes that usually change concentrations (von Schiller *et al.*, 2018), thereby resulting in contrasting nutrient fractions that are moved downstream by water discharge.

Nowadays it is hard to envisage the notion of a "pristine" environment. The Webster dictionary (1999) states that this means "untouched" in its third entry. In Spanish, the adjective "prístino" means "ancient, former, primitive, original" (Real Academia Española, 2014). Regarding catchments, "pristine" would mean no land use change, no agriculture, no cattle, no urban entities, no water channels and reservoirs, no human use and so on if the English meaning is followed, whereas it would mean that the catchment occurs in its primal state if we follow the Spanish definitions. Is there any site such as this in developed countries? Clearly, a new definition, adapted to current environmental features of the XXI century, is deserved. As an operative definition, "pristine" environments could be those where the habitats are free from any obvious signs of human activity and threats (excluding threats such as global warming as we assume that, those affect any environment worldwide).

Bearing in mind this operative definition it is well known that nutrients in pristine catchments experience large variability, both amongst sites and catchments (Temnerud and Bishop, 2005). Hence, one could wonder what environmental fluctuations could drive nutrient export and variability in pristine catchments, once human impact –other than climate change– are ruled out due to their very low significance. This leads us to the concentration-discharge relationship and its controlling factors (Evans and Davies, 1998), which have to be understood to provide insightful explanations for nutrient export downstream.

There are very few Iberian studies on stream nutrient export. Some deal with largely "pristine" basins (Ávila, 1989; Bernárdez *et al.*, 2013), whereas others report human impact on more complex catchments (Álvarez Cobelas *et al.*, 2010; Álvarez-Cobelas, 2019). Furthermore, long-term export studies have rarely been carried out in Spain in spite of the fact that there are official data available on discharge and nutrient contents which could encourage the calculation of export fluxes. They could be useful to ascertain if the export is affected by land-use changes and/or climatic features (either associated with global warming or not), but are largely neglected at present (see some preliminary information in Álvarez-Cobelas, 2019). The usefulness of pristine environments can certainly aid in clarifying natural effects from climate change effects on any environmental process, such as nutrient export, because the signals are not blurred by other man-made impacts. Our results can be used as a baseline when searching for changes in the future, whether resulting from global change or not.

The aim of this research has been to study the organic carbon, nitrogen and phosphorus export from two largely, undisturbed Mediterranean basins of the Iberian Peninsula, where this type of catchment is the exception rather than the rule. It has been undertaken in summer to avoid ice-melt and rainfall dilution effects on both discharge and nutrient flux. These basins share roughly the same climatic and geological conditions. Furthermore, using official data we have estimated long-term (1982-2010) total nitrogen and phosphorus export from three large sub-catchments in the area, which have not been subject to land use change since records have been available, in an attempt to relate this export with climate features. This endeavour may reveal whether export from pristine catchments could reflect global warming.

Study site

The Cuenca mountain range (Serranía de Cuenca) is located central-eastern Spain. It encompasses three areas at higher, middle and lower latitudes and also differing in altitude, which are mostly drained by two basins: the Tajo basin, which flows to the Atlantic Ocean, and the Júcar basin, which drains to the Mediterranean Sea. Both areas comprise 3,588 km^2 and are located at the NE part of the province of Cuenca, on the border of the provinces of Teruel and Guadalajara. The altitude ranges are 1,000-1,800 m.a.s.l., whereas the average slopes are 4-20%.

Underlying bedrocks are mostly Mesozoic llimestones with karst landscapes prevailing; tufa barrages are very common in streams. While the rainfall amounts to 950-1,110 mm/y, the average air temperature is within the range of 9.6-11.2 °C for the 1980-2014 period, the area thus being considered a Mediterranean cold climate which entails both a *Dsb* climate (cold with temperate dry summers) in the upper areas and a *Csb* climate (temperate with dry or temperate summers) in the lower altitudes, *sensu* Köppen classification (AEMET, 2011). Most soils are mollisols, following the classification by the Soil Survey Staff (1999).

The role of groundwater could be certainly important at headwater sites because they largely feed streams, with purely rainfall apportionment being lower. Groundwater permeability is mostly due to karstification, but groundwater quantitative data, other than location and some chemical analyses (Guerra, 1999), are lacking. Furthermore, there are no studies on their quantitative effects for water availability and discharge.

Stream discharge experiences a strong seasonality and many small streams dry up in summer, but very few are gauged. Discharge of permanent streams ranged from 0.01 to 2.6 m^3/sec in August (unpublished data).

Landscapes are largely secondary forests in many of the studied watersheds (Table 1), being comprised by *Pinus nigra* and *Juniperus* spp. in the upper areas and oak trees *(Quercus ilex*) in the lower altitude areas. Croplands were important until the sixties surrounding towns and villages but have since become rangelands, mostly covered by herbs and shrubs

(*Rosa* spp.). Urban and agricultural pollution is negligible nowadays. Other land use (industry, crops) is almost absent. Ovine flocks are very scattered, but they are not very numerous. Therefore, the territory could be considered as largely pristine, were it not for the building of four reservoirs in the 1950s and 60s for different water use purposes (irrigation, hydropower) in the Guadiela, Cuervo and Júcar rivers. Two small dams were also built on the upper Guadiela river in the 1940s to collect enough water for a pipe line to supply a 1.52 MW hydropower plant, and another small dam has a small artificial beach upstream from the village of Cañizares on the Escabas river.

The area is largely depopulated (3.5 inhabitants/km^2 mostly living in small towns and villages) as a result of migration to eastern Spanish cities in the 1960s and 70s, which has resulted in a negative population growth down to -66% between 1950 and 1991 (Cava, 1994). Tourism is still scarce and mostly made up of people living in big Spanish cities, whose relatives grew up in the area long ago, coming in the summer.

Further environmental information on the area can be found in Cava (1994), Mayoral (2011) and Gómez Serrano and Mayoral (2013).

Materials and methods

All the sampling was undertaken within two weeks in August, 2017. Only 27 stream sites had running water at that time, 14 were chosen in the Alto Tajo basin and 13 in the Alto Júcar basin (Table 2, Fig. 1), of which at least 7 sites were entirely pristine (*i.e.* without towns, croplands or reservoirs). Each one was visited only once. Discharge might have remained largely unchanged for two weeks because rainfall was absent in this period. Since there were no gauges, at each sampling station a cross-section was estimated by planimetry, and the discharge was measured three times at a central point in the field using a FLOW Global Water FP 101 probe. Both types of measurements enabled us to estimate the instantaneous discharge. Water temperature, dissolved oxygen, pH and conductivity were measured with ODO Yellow Springs and CRISON portable equipment. Dissolved oxygen was also used as a surrogate for biological activity, which could be important if much higher than saturation because it is well known that such a signal is the outcome of reaeration by atmospheric oxygen and the oxygen input evolved through plant photosynthesis within the stream channel.

Chemical analyses using APHA (2005) procedures were undertaken shortly after water collection. Nitrogen (nitrate, nitrite and ammonia) and SRP compounds were measured with a Seal-3 auto-analyzer, whereas organic carbon (total, TOC hereafter, and dissolved, DOC hereafter) and total nitrogen were with a TOC-V_{CSH} Shimadzu equipment. We lack data on the sensitivity of these apparatuses. Raw samples were digested with strong acids to mineralize all phosphorus forms to render total phosphorus as SRP, which was measured as above. The precision (reproducibility, *i.e.* as standard error of the mean, which can be reported as percentage of the latter) of all measurements were 0.22%, 0.06% and 0.006% for carbon, nitrogen and phosphorus, respectively. The limits of detection were 0.07 mg C/L, 0.03 mg N/L and 0.002 mg P/L for carbon, nitrogen and phosphorus, respectively.

Loadings were estimated by multiplying nutrient concentration discharge times and were reported on a per day basis assuming that instantaneous data did not change much over a day. These data were converted to fluxes by dividing them by the area of upstream catchment. Retention or export is usually estimated as percentages of flux between an upper site and a lower site value in a given stream. However, this does not enable the comparison of such values with each other because it does not take the length of the channel into account, which usually differs between both sites. Therefore, we here suggest what we call "length-scale net retention" (or export) in the following way

$$LSNR = (Nutflux_L - Nutflux_U)/D$$

where LSNR is measured in ng/km/d, $Nutflux_L$ is the nutrient flux (either OC, N or P, as measured in ng/km^2/d) in the lower site of a given stream, $Nutflux_U$ is the nutrient flux (either OC, N or P) in the upper site of a given stream, and D is the distance (km) between both sites.

Data about the catchment area, average slopes and land use within catchments were ascertained from the SIGPAC (Sistema de Información Geográfica de la Política Agraria Comunitaria) facility (www.sigpac.jccm.es) with ARGIS v10 using a Digital Elevation Model (DEM) with 25 m of resolution (MDT25, IGN). Nevertheless, the lack of detailed data leads to some shortcomings when these catchments need to be adequately described from an environmental viewpoint. Despite their interest as pristine areas, information on their surficial and groundwater hydrology, soils, fluvial network features (form, hypsometry, slopes...) and detailed land use are still rather poor, and hence they cannot be used to suggest more accurate relationships between nutrient export and its explanative catchment variables.

Furthermore, the long-term export of total nitrogen and phosphorus was estimated for three catchments, two in the Upper Tajo basin (the Escabas river at Priego,

	Catchment number	Overall catchment (ha)	Partial catchment (ha)	Cropland or rangeland (ha)	Forests (ha)	Cropland or rangeland (%)	Forests (%)	Average slope (%)	Village upstream	Retention upstream
Júcar basin										
Algarra	23	75		10	65	13	87	12	No	No
Cabriel-1	19	11000		200	10800	2	98	15	Yes	No
Cabriel-2	20	11450	450	600	10850	5	95	7	Yes	No
Cabriel-3	21	13950	2500	800	13150	6	94	5	Yes	No
Cabriel-4	22	21198	4400	1865	19333	9	91	4	Yes	No
Júcar-1	12	185		0	185	0	100	10	No	No
Júcar-2	13	2385	2200	2100	285	88	12	5	Yes	No
Júcar-3	14	4000		2300	1700	58	43	3	Yes	Yes
Mayor-1	17	1600		1400	200	88	13	10	Yes	No
Mayor-2	18	2805	800	2600	205	93	7	4	Yes	No
Laguna	15	382	300	300	82	79	21	12	Yes	No
Tejadillos	16	2282	1900	500	1782	22	78	8	No	No
Vencherque	24	650		50	600	8	92	15	Yes	No
Tajo basin										
Alcantud	25	1421		1300	121	91	9	12	Yes	No
Cuervo-1	4	116		0	116	0	100	20	No	No
Cuervo-2	5	331	215	215	116	65	35	7	Yes	No
Cuervo-3	6	1920	1589	215	1705	11	89	5	Yes	No
Cuervo-4	7	2848	928	265	2583	9	91	6	Yes	Yes
Escabas-1	8	660		0	660	0	100	15	No	No
Escabas-2	9	4160	3500	500	3660	12	88	10	Yes	No
Escabas-3	10	6030	1870	1000	5030	17	83	8	Yes	No
Escabas-4	11	31030	25000	26000	5030	84	16	3	Yes	No
Guadazaón	26	500		50	450	10	90	15	N	No
Guadiela-1	1	1425		25	1400	2	98	10	N	No
Guadiela-2	2	3445	2020	1200	2245	35	65	7	Yes	Yes
Guadiela-3	3	5850	2000	1225	4625	21	79	5	Yes	Yes
Masegar	27	405		300	105	74	26	10	No	No

Table 1. Environmental features of studied catchments in summer 2017 in the Serranía de Cuenca. See site description and location in Table 2 and Figure 1. Data gathered from the SIGPAC facility (www.sigpac.jccm.es).

Tabla 1. *Características ambientales de las cuencas estudiadas en el verano de 2017 en la Serranía de Cuenca. Véanse su descripción y localización en la Tabla 2 y la Figura 1. Los datos se han obtenido de la plataforma del Sistema de Información Geográfica de la Política Agraria Comunitaria (www.sigpac.jccm.es).*

station 3045, and the Guadiela river at Alcantud, station 3041) and one in the Upper Júcar basin (the Cabriel river at Pajaroncillo, station 8090, see their locations in Table 2). Discharge and water quality data which have been gathered since 1982 by the Tajo and Júcar Water Authorities (www.chjucar.es; www.chtajo.es) were used. Chemical data, however, are affected by two shortcomings. Firstly, since concentrations are quite low most of the time, the Water Authorities do not provide true concentration data, but rather an upper limit suggested by the limit of detection of the chemical method involved, these limits being 0.10 mg NO_3/L, 0.03 mg NH_4/L and 0.02 mg PO_4/L. We therefore decided to use the detection limits sometimes provided by these authorities to

	Catchment number	Location	Lat (° N)	Long (° W)
Júcar basin				
Algarra	23	Algarra river (Algarra upstream)	40.000508	1.440882
Cabriel-1	19	Cabriel river (Salvacañete downstream)	40.09516	1.508298
Cabriel-2	20	Cabriel river (Alcalá de la Vega upstream)	40.034289	1.510873
Cabriel-3	21	Cabriel river (Boniches downstream)	39.983422	1.642826
Cabriel-4	22	Cabriel river (near Villar de Humo)	39.840616	1.663295
Júcar-1	12	Júcar river (close to its spring)	40.364068	1.828773
Júcar-2	13	Júcar river at Huélamo	40.279913	1.813438
Júcar-3	14	Júcar river at Uña	40.222942	1.981038
Mayor-1	17	Mayor river (Cañete upstream, Tejadillos river downstream)	40.052905	1.631152
Mayor-2	18	Mayor river (Cañete downstream)	40.009881	1.657759
Laguna	15	Laguna river (Marquesado lake downstream)	40.169487	1.673149
Tejadillos	16	Tejadillos river (close to Cañete)	40.066131	1.622912
Vencherque	24	Vencherque river at Villar del Humo	39.868202	1.624728
Meteorological station		Boniches	39.585600	1.372120
Gauging station		Cabriel river at Pajaroncillo	39.939660	1.711129
Tajo basin				
Alcantud	25	Alcantud river (Alcantud downstream)	40.573255	2.326328
Cuervo-1	4	Cuervo river spring	40.428982	1.886140
Cuervo-2	5	Cuervo river at Codorno valley	40.442603	1.951648
Cuervo-3	6	Cuervo river (Santa María del Val upstream)	40.501225	2.038051
Cuervo-4	7	Cuervo river (Solán de Cabras upstream	40.511819	2.127287
Escabas-1	8	Escabas river at Tejadillos	40.394479	1.982634
Escabas-2	9	Escabas river (Cañamares upstream)	40.471048	2.220357
Escabas-3	10	Escabas river (Cañamares downstream)	40.447017	2.247966
Escabas-4	11	Escabas river (before joining Guadiela river)	40.468329	2.377197
Guadazaón	26	Guadazaón river (Valdemoro Sierra upstream)	40.108318	1.771501
Guadiela-1	1	Guadiela river at Beteta	40.575957	2.077311
Guadiela-2	2	Guadiela river (Puente Vadillos upstream)	40.533916	2.147750
Guadiela-3	3	Guadiela river upstream La Ruidera reservoir (close to Alcantud)	40.509253	2.321414
Masegar	27	Masegar river (El Tobar lake downstream)	40.550353	2.062835
Meterological station		Cañizares	40.314900	2.090020
Gauging station		Escabas river at Priego	40.442975	2.311364
Gauging station		Guadiela river at Alcantud	39.939660	1.711129

Table 2. Studied watersheds in the Serranía de Cuenca in summer 2017. See also Figure 1. Locations of meteorological and gauging stations for the long-term study are also given.

Tabla 2. *Cuencas estudiadas en la Serranía de Cuenca en el verano de 2017. Véase también la Figura 1. Se incluye también la localización de las estaciones meteorológicas y de aforos usadas en el estudio a largo plazo.*

All basins (N = 27)	TOC	DOC	POC	Nitrate	Nitrite	Ammonia	Dissolved nitrogen	Particulate nitrogen	Organic nitrogen	Total nitrogen	SRP	Total phosphorus
Average	1.184	1.052	0.132	0.355	0.014	0.048	0.510	0.034	0.128	0.545	0.006	0.009
SD	0.689	0.703	0.122	0.362	0.015	0.044	0.462	0.051	0.151	0.486	0.006	0.013
CV	58	67	92	102	102	93	91	150	119	89	103	150
Min	0.488	0.365	0.003	0.002	0.007	0.006	0.072	0.001	0.000	0.093	0.000	0.000
Max	3.445	3.332	0.435	1.272	0.069	0.175	1.719	0.262	0.591	1.785	0.024	0.069
Júcar basin (N = 13)												
Average	1.021	0.886	0.136	0.444	0.018	0.062	0.655	0.020	0.152	0.675	0.007	0.008
SD	0.710	0.737	0.132	0.347	0.020	0.055	0.498	0.023	0.196	0.514	0.007	0.006
CV	70	83	97	78	109	89	76	114	129	76	96	74
Min	0.488	0.365	0.004	0.069	0.007	0.019	0.166	0.001	0.000	0.178	0.002	0.003
Max	3.178	3.109	0.376	1.136	0.069	0.175	1.719	0.066	0.591	1.785	0.024	0.019
Tajo basin (N = 14)												
Average	1.335	1.206	0.128	0.273	0.011	0.035	0.376	0.048	0.105	0.423	0.005	0.010
SD	0.657	0.658	0.117	0.368	0.008	0.028	0.399	0.066	0.096	0.442	0.005	0.017
CV	49	55	91	135	68	81	106	139	91	104	110	181
Min	0.741	0.647	0.003	0.002	0.007	0.006	0.072	0.003	0.019	0.093	0.000	0.000
Max	3.445	3.332	0.435	1.272	0.030	0.095	1.370	0.262	0.377	1.433	0.017	0.069

Table 3. Average and variability of nutrient concentrations in the studied catchments in summer 2017. All units are either mg C/L, mg N/L or mg P/L. CV: coefficient of variation, DOC: dissolved organic carbon, max: maximal value, min: minimal value, POC: particulate organic carbon, SD: standard deviation, SRP: soluble reactive phosphorus, TOC: total organic phosphorus.

Tabla 3. *Promedios y variabilidad de las concentraciones de nutrientes en las cuencas estudiadas en el verano de 2017. Todas las unidades están en mg C/L, mg N/L y mg P/L. CV: coeficiente de variación, DOC: carbono orgánico disuelto, max: valor máximo, min: valor mínimo, POC: carbono orgánico particulado, SD: desviación típica, SRP: fósforo reactivo soluble, TOC: carbono orgánico total.*

represent chemical concentrations when true concentration data were not supplied. Secondly, more often than not total nitrogen and phosphorus official data were unavailable for these catchments. We chose to estimate them by multiple regression using our databases for each basin as raw data inputs. So for the Júcar basin the equations were the following

$$\text{Total N} = 0.064 + 1.377*[\text{N-NO}_3]$$
$$\text{Adj } R^2 = 0.85 \quad p < 0.000002 \quad n = 13$$
$$\text{Total P} = 0.013*\text{Discharge} + 0.01 * [\text{N-NO}_3] + 0.003 * [\text{N-NH}_4] - 0.174 * [\text{P-SRP}] - 0.002$$
$$\text{Adj } R^2 = 0.68 \quad p < 0.008 \quad n = 13$$

And for the Tajo basin they were

$$\text{Total N} = 1.14 * [\text{N-NO}_3] + 2.56 * [\text{N-NH}_4]$$
$$\text{Adj } R^2 = 0.97 \quad p < 0.000001 \quad n = 14$$
$$\text{Total P} = 3.016 * [\text{P-SRP}] - 0.004 \text{ Adj } R^2 = 0.70 \quad p < 0.00011 \quad n = 14$$

where all the concentration units are mg/L and the discharge unit is m^3/sec, with SRP being the soluble reactive phosphorus.

Since the frequency of chemical data is very changeable throughout the years, we decided to estimate a yearly flux by averaging chemical data for each year involved and then multiplying them by the sum of all the monthly discharge data. Thus we avoided the humble and largely inaccurate task of estimating the missing data of the chemical concentrations. The Spanish Meteorological Agency (AEMET hereafter) provided long-term data on rainfall and air temperatures for the sites close to the studied catchments which were Cañizares (Central Vadillo, station 3044 of AEMET; see the geographical coordinates in Table 2) in the Upper Tajo catchment and Boniches (station 8213 of AEMET) in the Upper Júcar catchment, both of which are approximately located at the centre of each territory.

	TOC	DOC	POC	Nitrate	Nitrite	Ammonia	Dissolved nitrogen	Particulate nitrogen	Organic nitrogen	Total nitrogen	SRP	Total phosphorus
All basins (N = 27)												
Average	3.788	3.366	0.422	1.136	0.046	0.153	1.633	0.110	0.409	1.743	0.018	0.028
SD	2.203	2.249	0.390	1.158	0.047	0.142	1.480	0.164	0.484	1.555	0.019	0.042
CV	58	67	92	102	102	93	91	150	118	89	103	150
Min	1.561	1.169	0.010	0.006	0.022	0.019	0.229	0.002	0.000	0.299	0.000	0.000
Max	11.024	10.662	1.393	4.070	0.221	0.560	5.501	0.838	1.891	5.712	0.077	0.221
Júcar basin (N = 13)												
Average	3.268	2.834	0.435	1.420	0.057	0.198	2.096	0.064	0.485	2.160	0.023	0.024
SD	2.273	2.357	0.421	1.112	0.062	0.176	1.592	0.073	0.628	1.646	0.022	0.018
CV	70	83	97	78	109	89	76	114	129	76	96	74
Min	1.561	1.169	0.014	0.221	0.022	0.061	0.532	0.002	0.000	0.569	0.006	0.010
Max	10.170	9.949	1.204	3.635	0.221	0.560	5.501	0.211	1.891	5.712	0.077	0.061
Tajo basin (N = 14)												
Average	4.271	3.861	0.411	0.872	0.036	0.111	1.203	0.152	0.336	1.355	0.014	0.031
SD	2.102	2.107	0.375	1.177	0.024	0.090	1.275	0.212	0.306	1.414	0.016	0.056
CV	49	55	91	135	68	81	106	139	91	104	110	181
Min	2.371	2.070	0.010	0.006	0.022	0.019	0.229	0.009	0.060	0.299	0.000	0.000
Max	11.024	10.662	1.393	4.070	0.096	0.304	4.384	0.838	1.206	4.586	0.054	0.221

Table 4. Average and variability of nutrient export in the studied catchments in summer 2017. All units are either mg $C/m^2/d$, mg $N/m^2/d$ or mg $P/m^2/d$. Abbreviations as in Table 3.

Tabla 4. *Promedios y variabilidad de la exportación de nutrientes por las cuencas estudiadas en el verano de 2017. Todas las unidades figuran en mg C/m^2/día, mg N/m^2/día ó mg P/m^2/día. Las abreviaturas, como en la Tabla 3.*

	TOC	DOC	POC	NO_3	NH_4	Org-N	TN	SRP	TP
Cabriel	0.0281	0.0332	-0.0051	-0.0211	-0.0094	0.0006	-0.0304	-0.0004	-0.0001
Cuervo	0.0331	0.0157	0.0174	-0.0159	-0.0018	0.0062	-0.0114	0.0001	0.0002
Escabas	-0.0181	0.0138	-0.0319	0.1014	-0.0024	0.0067	0.1057	-0.0002	-0.0003
Guadiela	0.0907	0.0907	0.0000	0.0136	-0.0011	0.0166	0.0293	-0.0001	0.0000
Júcar	0.0870	0.0820	0.0050	-0.0103	-0.0001	0.0090	-0.0012	-0.0001	0.0002
Mayor	-0.2550	-0.0302	-0.2248	0.4224	0.0416	-0.0211	0.4653	0.0064	0.0064
Laguna-Tejadillos	-0.0674	-0.0945	0.0270	0.0869	0.0021	-0.0014	0.0873	0.0002	0.0002

Table 5. Stream length-scaled, net retention (values lower than 0) or export (>0) of nutrient fluxes in the main Serranía de Cuenca basins in summer 2017. Abbreviations as in Table 3. All units as ng/km/d.

Tabla 5. *Retención neta (valores inferiores a 0) o exportación neta (>0), divididas ambas por la longitud del cauce, de los flujos nutritivos en las principales subcuencas de la Serranía de Cuenca en el verano de 2017. Las abreviaturas, como en la Tabla 3. Todas las unidades, en ng/km/d.*

Our calculations spanned the years 1982-2010, but some in-between years lacked data on either the chemistry or the discharge. From 2011 onwards the data on the chemistry were either more scattered than before and/or they did not exceed detection limits and hence they were not chosen. In order to reduce the uncertainty of yearly estimations arising from both the poor temporal resolution of data and the low data number of total nutrients, we have also estimated an error value for each yearly data in each catchment by assuming an additive model of both the discharge and the nutrient errors (Taylor, 1997).

The statistical analyses to deal with raw data and searching for relationships amongst the variables were the Spearman correlations, power regressions, U tests (Siegel and Castellan, 1988) and principal component analyses (PCA hereafter; Legendre and Legendre, 2012). Power regressions between discharge and concentrations were estimated because it is the usual procedure when initially exploring such relationships (Evans and Davies, 1998). They were calculated with the Statistica 7.0 package. In order to fully understand the acknowledged multifactorial nature of environmental control on nutrient export (Johnson *et al.*, 1997), PCAs were calculated on nutrient fluxes (total organic carbon, dissolved organic carbon and particulate organic carbon, nitrate, ammonia, particulate nitrogen, organic nitrogen, total nitrogen, soluble reactive phosphorus, total phosphorus) and environmental variables (catchment area and average slope, discharge, fraction of crop and rangeland use, fraction of forest land use, water temperature, dissolved oxygen as percentage saturation, pH and conductivity as a surrogate of stream salinity) for the whole data set and also splitting the Júcar and Tajo sub-catchments. PCA analyses were undertaken using the PAST package (Hammer *et al.*, 2001).

Results

Environmental conditions

The commonplace view that some environmental conditions, such as rainfall and discharge in previous months, may influence nutrient export has led us to report them here. In fact, official data on rainfall and discharge in the previous year to our 2017 sampling show that they were much lower than long-term averages in the Upper Júcar and Upper Tajo catchments. For example, while the latter were 48.66 ± 42.94 mm/month and 9.76 ± 9.79 hm^3/month for rainfall and discharge, respectively, for the Upper Cabriel catchment, averages for the previous year to our sampling (Sept 2016-Aug 2017) were 34.15 ± 25.73 mm/month and 4.99 ± 1.51 hm^3/month, respectively. The same pattern occurred in the other three gauging stations and the single one for rainfall measurement. When the rainfall and discharge of the previous months to our sampling are plotted, discharge fluctuations happened to be less variable than rainfall fluctuations in the four stations where official data are available (Fig. 2). In the Upper Júcar catchment there were no statistically significant relationships between rainfall and discharge in the year before our sampling ($p > 0.05$), whereas there were weak relationships in the two Upper Tajo catchment stations ($R^2 = 0.20$ and 0.21 for the Escabas and Guadiela sub-catchments, $p < 0.05$). One-month lagged relationships explained even lower fractions of variability ($R^2 < 0.10$). Discharge showed lower variability than rainfall for the four sub-catchments involved (rainfall CVs = 72-75%, discharge CVs = 24-45%). Nevertheless, changing catchment features (occurrence of reservoirs, land use, groundwater inputs) might be responsible for the different behaviour of the rainfall-discharge relationships in both the upper catchments.

In situ stream measurements of physico-chemical features provided a very conservative picture across sub-catchments with low variability (CVs lower than 25%), except for discharge (CV = 128%) which ranged 0.009-2.571 m^3/sec. Water temperature ranged between 11.7-23.0 ºC, the lowest value belonging to the upper sites receiving groundwater. Dissolved oxygen almost always displayed undersaturation (average ± 1 SD = 91 ± 22%; range = 71-184%); only a couple of sites showed weak supersaturation and a third one experienced strong supersaturation, the latter arising from high local photosynthesis by charophytes and periphyton. So these oxygen features suggest a low importance of in-stream biological processes in most of the sub-catchments studied. Whilst the pH was alkaline throughout (range = 7.85-8.59), water conductivity was not very high in these carbonated waters (range = 400-1454 µS/cm). Despite the suspected importance of groundwater quantity and quality to in-stream nutrient contents and fluxes, no data were available.

Short-term, summer nutrient export and its controlling factors

Average nutrient concentrations were suggestive of very clean streams in most instances (Table 3). Nitrate and DOC were the main fractions of overall nitrogen and organic carbon, respectively, organic nitrogen roughly being one fourth (27 ± 21%) of the total nitrogen for all streams. Variability of total nitrogen and phosphorus was higher in the Tajo catchment, but DOC variability was higher in the Júcar catchment (Table 3).

Organic C, total N and total P export ranged 1.56-11.02 mg C/m^2/d, 0.30-5.71 mg N/m^2/d and 0-0.221 mg P/m^2/d, respectively (Table 4). Since only one assessment of discharge was undertaken, flux variability traced that of concentration. Length-scaled net retention (*i.e.* smaller fluxes in lower sites than in upper sites of the same stream) was highly changeable and variable amongst both the nutrients and the catchments (Table 5). Fluxes often showed net retention, such as that which occurred with either the nitrate flux in the Cabriel, Cuervo and Júcar rivers or the ammonia flux in the Cabriel, Cuervo, Escabas, Guadiela and Júcar rivers (Table 5). Whilst the same river experienced net retention for organic carbon and nitrogen, it yielded net export for nitrate, ammonia, total N, SRP and total P fluxes, as shown by the Mayor river values (Table 5).

Catchment size was unrelated with nutrient concentrations ($p > 0.10$). Entirely pristine sites ($n = 7$) were less variable for DOC and total P concentrations than the remaining sites (U Mann-Whitney test, $p = 0.053$ and 0.077, respectively), but the other chemical variables did not show any differences in variability between pristine and not entirely pristine sites ($p > 0.10$).

For the whole data set, there were no statistically significant relationships between discharge and nutrient concentrations ($p > 0.10$). Nearby towns or villages did not appear to affect stream nutrient concentrations because their occurrence neither increased nor decreased those contents (U Mann-Whitney test, $p > 0.10$). However, water retention by lakes and reservoirs impinged on NH_4, NO_3, POC, DOC and total N concentrations and fluxes (U Mann-Whitney test, $p = 0.052\text{-}0.070$) by decreasing their variability downstream. The same was true for SRP flux, but the total P flux remained unaffected by either water retention or towns or villages located upstream ($p > 0.10$).

Catchment surface area was unrelated with any nutrient flux ($p > 0.10$). Only catchment areas of croplands were related with nitrate flux ($R^2 = 0.47$; $p = 0.006$); the remaining fluxes did not appear to be controlled by either catchment area or land use. Interestingly, forested areas were also unrelated with any nutrient flux downstream ($p > 0.10$). Total organic C fluxes were dependent upon DOC flux ($R^2 = 0.98$, $p = 0.00005$), and nitrate flux was inversely related with organic N flux ($R^2 = 0.55$, $p = 0.003$).

The first three factors of PCA on the whole data set explained 59.4% of the whole variability, with the first Factor encompassing more 30% of overall variance (Table 6). Nutrient fluxes of the whole dataset were related with Factor I, and land use (%crops and rangeland and %forests) and water conductivity as well. Whilst Factor II was explained by catchment area, slope and discharge, Factor III was by land use again, albeit with stronger correlations than Factor I (Table 7).

When the Upper Júcar and Upper Tajo catchments were considered separately, discharge (Q) was found to be related with ammonia and total P in Júcar ($R^2 = 0.37$ and 0.56, $p = 0.026$ and 0.003, respectively), whereas it was related with nitrate in Tajo ($R^2 = 0.30$, $p = 0.041$). Power functions, however, were only statistically significant for ammonia in the latter catchment ($[N\text{-}NH_4] = 0.015 * Q^{-0.31}$; $R^2 = 0.24$, $p = 0.08$), the remaining concentration variables did not show any statistically significant power relationship with discharge for any sub-catchment. Catchment area did not control any concentration in the former, but nitrate appeared to be dependent on that in the Tajo catchment ($R^2 = 0.59$, $p = 0.001$). The extent of forested area was unrelated with any nutrient content or flux ($p > 0.10$).

After the splitting of the larger sub-catchments, the first three Factors explained 65.6% and 77.5% of overall variability, respectively. In both analyses the first Factor explained more than 30% of total variance (Table 6). Most fluxes were related to Factor I in the Upper Júcar basin, which was mostly explained by in-stream salinity; Factor II was related with NH_4 ant total P fluxes, catchment average slope and discharge whereas Factor III could represent land use. In the Upper Tajo basin, most fluxes were associated to Factor I, which was mostly explained by land use (crops, rangelands, forests); Factor II was somewhat related with NH_4 flux, catchment average slope, discharge and water conductivity whereas Factor III could represent the in-stream abiotic environment (water temperature, dissolved oxygen and pH; Table 7).

Long-term nutrient export and its control

Annual N export from Tajo major sub-catchments showed a bimodal pattern over time in the long-term (1982-2010), but that of P was fluctuating throughout the study period (Figs. 3-4). In the Júcar basin, export downstream from Cabriel showed several peaks for both nutrients (Fig. 5). Whilst concentrations of both dissolved and total nutrients and the discharge were not statistically related for all data pairs available ($n > 100$, $p > 0.10$) in Guadiela and Cabriel sub-catchments, total N was statistically significantly related with discharge in the Escabas sub-catchment ($[TN] = 5.22 * Q^{-0.69}$; $R^2 = 0.24$, $n = 105$, $p = 0.0003$). Since no data on organic C concentration were available for any sub-catchment, their flux calculations could not be carried out.

Annual rainfall appeared to partly control annual P export fluxes in the three sub-catchments studied ($R^2 = 0.16$, 0.17 and 0.42 for Guadiela, Escabas and Cabriel, respectively; $p = 0.012\text{-}0.0003$) through control of the

	All catchments	Upper Júcar catchment	Upper Tajo catchment
Factor I	30.17	32.10	42.32
Factor II	17.28	19.43	24.54
Factor III	11.93	14.06	10.69
Factor IV	9.45	10.62	6.31
Factor V	8.03	7.12	5.41
Cumulative	76.86	83.33	89.27

Table 6. Variance (as percentage of the whole variability) explained by the first five factors of PCA analyses when carried out on the whole data set (environmental and nutrient export data) of Serranía de Cuenca, collected in summer 2017, and splitting the Upper Júcar and Upper Tajo catchments.

Tabla 6. *Varianza (como porcentaje de la variabilidad total) explicada por los cinco primeros factores de los análisis de componentes principales, realizados sobre el total de los datos ambientales y de exportación de nutrientes de las cuencas de la Serranía de Cuenca en verano de 2017, o bien separando las subcuencas del Alto Júcar de las del Alto Tajo.*

	All catchments			Upper Júcar catchment			Upper Tajo catchment		
	Factor I	Factor II	Factor III	Factor I	Factor II	Factor III	Factor I	Factor II	Factor III
Catchment area	0.228	0.73	0.31	0.111	0.580	-0.407	0.279	0.903	-0.149
% Crops & rangeland	0.431	0.331	-0.73	-0.408	0.154	0.858	0.865	0.181	-0.153
%Forests	-0.415	-0.33	0.729	0.407	-0.155	-0.858	-0.865	-0.181	0.154
%Slope	-0.048	-0.759	0.254	0.297	-0.666	-0.281	-0.149	-0.677	-0.560
Discharge	0.244	0.812	0.142	0.088	0.938	0.057	0.222	0.844	-0.061
Water temperature	0.079	0.392	-0.331	-0.062	0.225	0.045	0.169	0.406	0.598
O2 saturation	-0.214	-0.008	0.043	0.264	-0.076	-0.117	-0.291	0.0095	0.654
pH	0.211	0.473	-0.122	-0.052	0.456	0.412	0.232	0.414	0.702
Water conductivity	0.496	0.597	0.360	0.858	-0.047	0.321	0.259	0.889	-0.318
TOC export	0.741	-0.353	-0.215	0.674	-0.394	0.335	0.873	-0.341	0.167
DOC export	0.725	-0.333	-0.240	0.626	-0.466	0.384	0.875	-0.290	0.208
POC export	0.036	-0.069	0.176	0.132	0.471	-0.334	0.0029	-0.292	-0.234
NO3 export	0.788	0.199	0.282	0.745	-0.038	0.269	0.777	0.488	-0.249
NH4 export	0.399	-0.059	0.140	0.360	0.689	-0.082	0.575	-0.613	0.096
PON export	0.756	-0.343	-0.193	0.817	-0.002	-0.329	0.898	-0.299	-0.012
Org-N export	0.753	-0.135	0.459	0.902	0.104	-0.120	0.873	-0.066	0.053
TN export	0.876	0.103	0.372	0.899	0.109	0.134	0.886	0.349	-0.188
SRP export	0.814	-0.283	0.188	0.927	0.065	0.259	0.778	-0.422	0.088
TP export	0.715	-0.316	-0.333	0.380	0.789	0.069	0.860	-0.435	0.141

Table 7. Factor loadings (as correlation coefficients) of the first three factors of PCA analyses on environmental and nutrient export data when carried out on the whole data set and splitting the Júcar and Tajo catchments. Statistically significant values at the $P < 0.05$ level are underlined.

Tabla 7. *Cargas (medidas como coeficientes de correlación) de los tres primeros factores de los análisis de componentes principales realizados sobre el total de los datos ambientales y de exportación de nutrientes para las cuencas de la Serranía de Cuenca en verano de 2017 y separando las subcuencas del Alto Júcar de las del Alto Tajo. Se han subrayado los valores estadísticamente significativos al nivel de probabilidad del 5%.*

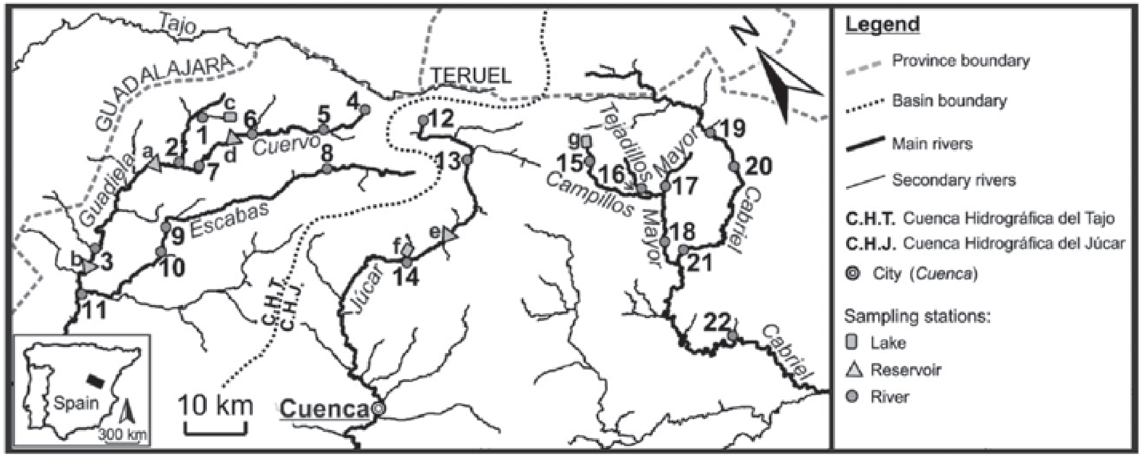

Figure 1. Location of main sampling sites. 1: Guadiela river at Beteta (Guadiela-1); 2: Guadiela river upstream Puente Vadillos (Guadiela-2); 3: Guadiela river upstream La Ruidera reservoir (close to Alcantud, Guadiela-3); 4: Cuervo river spring (Cuervo-1); 5: Cuervo river at Codorno valley (Cuervo-2); 6: Cuervo river, Santa María del Val upstream (Cuervo-3); 7: Cuervo river, Solán de Cabras upstream (Cuervo-4); 8: Escabas river at Tejadillos (Escabas-1); 9: Escabas river, Cañamares upstream (Escabas-2); 10: Escabas river, Cañamares downstream (Escabas-3); 11: Escabas river before joining Guadiela river (Escabas-4); 12: Júcar river close to its spring (Júcar-1); 13: Júcar river at Huélamo (Júcar-2); 14: Júcar river at Uña (Júcar-3); 15: Laguna/Campillos river, downstream Laguna del Marquesado town; 16: Tejadillos river close to Cañete; 17: Mayor river upstream Cañete (Mayor-1); 18: Mayor river, downstream Cañete (Mayor-2); 19: Cabriel river, downstream Salvacañete (Cabriel-1); 20: Cabriel river, upstream Alcalá de la Vega (Cabriel-2); 21: Cabriel river, downstream Boniches (Cabriel-3); 22: Cabriel river near Villar de Humo (Cabriel-4); a: Molino de Chincha reservoir; b: La Ruidera reservoir; c: El Tobar lake; d: La Tosca reservoir; e: La Toba reservoir; f: Uña lake; g: Marquesado lake. To make the picture clearer, other sites in smaller sub-catchments (sites 23-27) and further information can be found in Tables 1-2.

Figura 1. *Localización de las principales estaciones de muestreo. 1: río Guadiela en Beteta (estación Guadiela-1); 2: río Guadiela aguas arriba de Puente Vadillos (Guadiela-2); 3: río Guadiela aguas arriba del embalse de La Ruidera (cerca de Alcantud, Guadiela-3); 4: nacimiento del río Cuervo (Cuervo-1); 5: río Cuervo en el valle del Codorno (Cuervo-2); 6: río Cuervo, aguas arriba de Santa María del Val (Cuervo-3); 7: río Cuervo aguas arriba de Solán de Cabras (Cuervo-4); 8: río Escabas en Tejadillos (Escabas-1); 9: río Escabas aguas arriba de Cañamares (Escabas-2); 10: río Escabas aguas abajo de Cañamares (Escabas-3); 11: río Escabas antes de su confluencia con el río Guadiela (Escabas-4); 12: río Júcar, cerca de su nacimiento (Júcar-1); 13: río Júcar en Huélamo (Júcar-2); 14: río Júcar en Uña (Júcar-3); 15: río Laguna/Campillos aguas abajo del pueblo Laguna del Marquesado; 16: río Tejadillos cerca de Cañete; 17: río Mayor, aguas arriba de Cañete (Mayor-1); 18: río Mayor, aguas debajo de Cañete (Mayor-2); 19: río Cabriel aguas abajo de Salvacañete (Cabriel-1); 20: río Cabriel aguas arriba de Alcalá de la Vega (Cabriel-2); 21: río Cabriel aguas abajo de Boniches (Cabriel-3); 22: río Cabriel aguas abajo de Villar del Humo (Cabriel-4); a: embalse de Molino de Chincha; b: embalse de La Ruidera; c: laguna de El Tobar; d: embalse de La Toba; f: laguna de Uña; g: laguna del Marquesado. A fin de hacer la figura más nítida, las localizaciones de las cuencas de menor tamaño (estaciones 23-27) se citan en las Tablas 1-2, junto con información adicional.*

yearly discharge, which was dependent upon rainfall and its variability (R^2 = 0.12-0.48; p = 0.005-0.0002), but also on groundwater inputs as well, whose data were unfortunately lacking. Incidentally, yearly discharge was also statistically significantly related among sub-catchments (R^2 = 0.48-0.70; p = 0.0004-0.00006) in spite of the fact that the Guadiela catchment is regulated, but the other two are not. This fact would imply that all the watersheds behaved in a hydrologically similar manner. Yearly-averaged air temperature and its variability, whose long-term trends can be considered as another surrogate of global warming, did not show any statistically significant relationship with nutrient export in these catchments ($p > 0.05$).

Discussion

Short-term, summer nutrient export

Our results suggest that average nutrient concentrations in the Serranía de Cuenca streams are rather low and hence their waters appear to be very clean (Table 3), despite the fact that human population increases in summertime in the area along with a lowering of the discharge. More evidence of the pristine condition of these catchments is the fact that 44% of nutrient export variables in the seven bigger catchments show net retention (Table 5). In man-made impacted catchments the rule is net export throughout (Álvarez-Cobelas *et al.* 2008, 2009, 2012).

Since N:P ratios are always higher than 100 (by atoms), all studied stretches appear to be very phosphorus-limited (Dodds, 2006). The fraction of organic nitrogen content is high, as in other forested catchments (Pellerin *et al.*, 2006). The variability of concentrations change from one catchment to the other regarding nutrients, but also spanned one-to-three orders of magnitude concerning organic C, total P and total N, respectively (Table 3). Thus, despite the low concentrations their variability can be high (CVs usually around 100%), as also reported by studies in other headwater environments, roughly experiencing

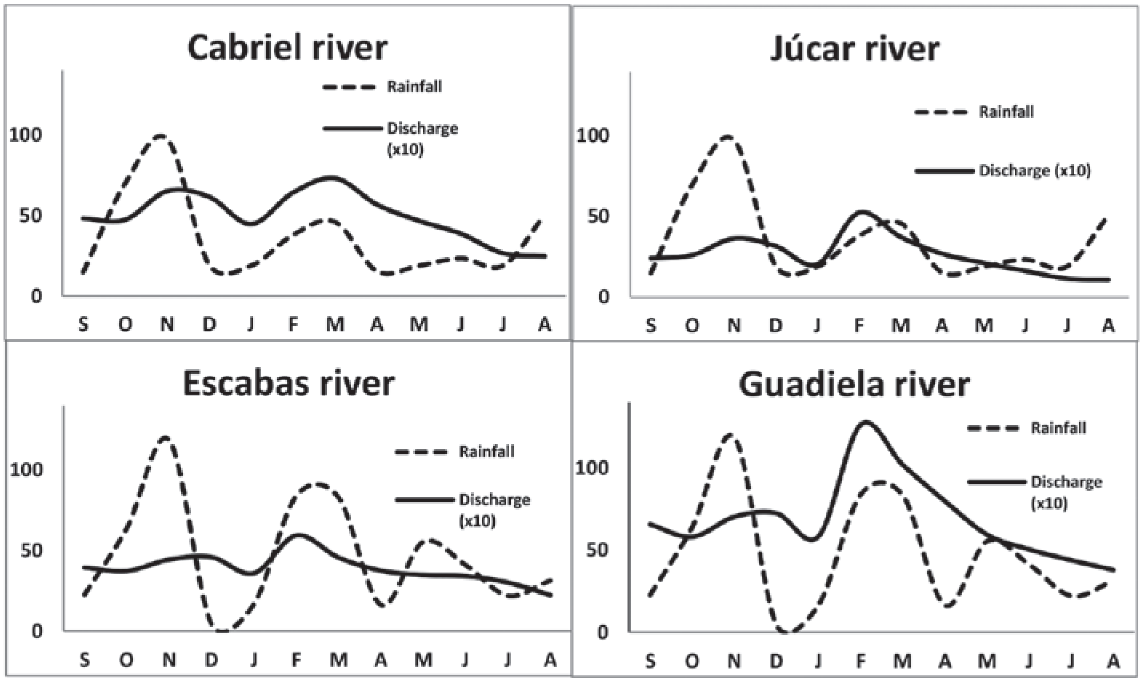

Figure 2. Monthly rainfall (mm/month) and discharge (hm³/month, notice the 10-fold enlargement) in the available official gauging and meteorological stations located in the Upper Júcar (upper panels) and Upper Tajo (lower panels) catchments from September 2016 (S) to August 2017 (A). Discharge stations are Cabriel at Pajaroncillo, Júcar at Huélamo (both run by the Júcar Water Authority), Escabas at Priego and Guadiela at Alcantud (both run by the Tajo Water Authority). There are only two meteorological stations per each large catchment, Boniches at Upper Júcar and Cañizares (Central Vadillos) at Upper Tajo; both are run by AEMET (the Spanish Meteorological Office). See their geographic coordinates in Table 2.

Figure 2. *Precipitación (mm/mes) y caudales (hm3/mes, nótese el cambio de escala) mensuales en las estaciones oficiales de aforos y meteorología disponibles en la zona de estudio del Alto Júcar (paneles superiores) y Alto Tajo (paneles inferiores) desde septiembre de 2016 a Agosto de 2017. Las estaciones de aforo son la del Cabriel en Pajaroncillo, la del Júcar en Huélamo (ambas mantenidas por la Confederación Hidrográfica del Júcar), la del Escabas en Priego y la del Guadiela en Alcantud (ambas mantenidas por la Confederación Hidrográfica del Tajo). Solo hay dos estaciones meteorológicas para cada una de las grandes subcuencas, la de Boniches en el Alto Júcar y la de Cañizares (Central Vadillos) en el Alto Tajo, las cuales son gestionadas por la AEMET. Véanse sus coordenadas geográficas en la Tabla 2.*

an inverse increase with catchment size (Lewis, 2002; Temnerud and Bishop, 2005), but such a relationship was not observed in our study. All these facts are interesting because they make one wonder about what natural sources of nutrient variability are operating in pristine catchments (differential export from terrestrial environments, in-stream metabolism...?) and at what spatial scales. However, we currently lack information to suggest environmentally sounding explanations for this at the catchment spatial scale of pristine environments.

Regarding the magnitudes of nutrient export downstream, they range from one- for organic C and total N to three orders of magnitude for total P (Table 4). Nevertheless these values are within the lower quartile of reported export worldwide (Alvarez-Cobelas *et al.*, 2008, 2009, 2012). Since all the studied catchments are karstic environments, where calcium deposition is a dominant process, the P export must certainly be lower than expected on landscape grounds because P retention is largely regulated by calcium deposition (Corman *et al.*, 2016). N export, however, is usually enhanced by carbonate deposition (Corman *et al.*, 2015), the karstic nature of these catchments thereby resulting in higher N:P ratios than those occurring in non-karstic streams. In fact, we have observed N:P ratios which exceed 100 (by atoms, see above) in most instances, these ratios being higher than those in the observations of Corman *et al.* (2016).

Entirely pristine sites (one fourth of the whole data set) are less variable for DOC and total P concentrations than the remaining sites, but in-stream sites close to towns and villages where concentrations have been measured do not appear to impinge on stream nutrient concentrations. Both facts suggest that entirely pristine sites damp out environmental variability of DOC and P export, and that the summertime human population (the highest in the year) exerts a negligible impact on nutrient concentrations in this mountain area.

For the whole data set, there are no statistically significant relationships between discharge and nu-

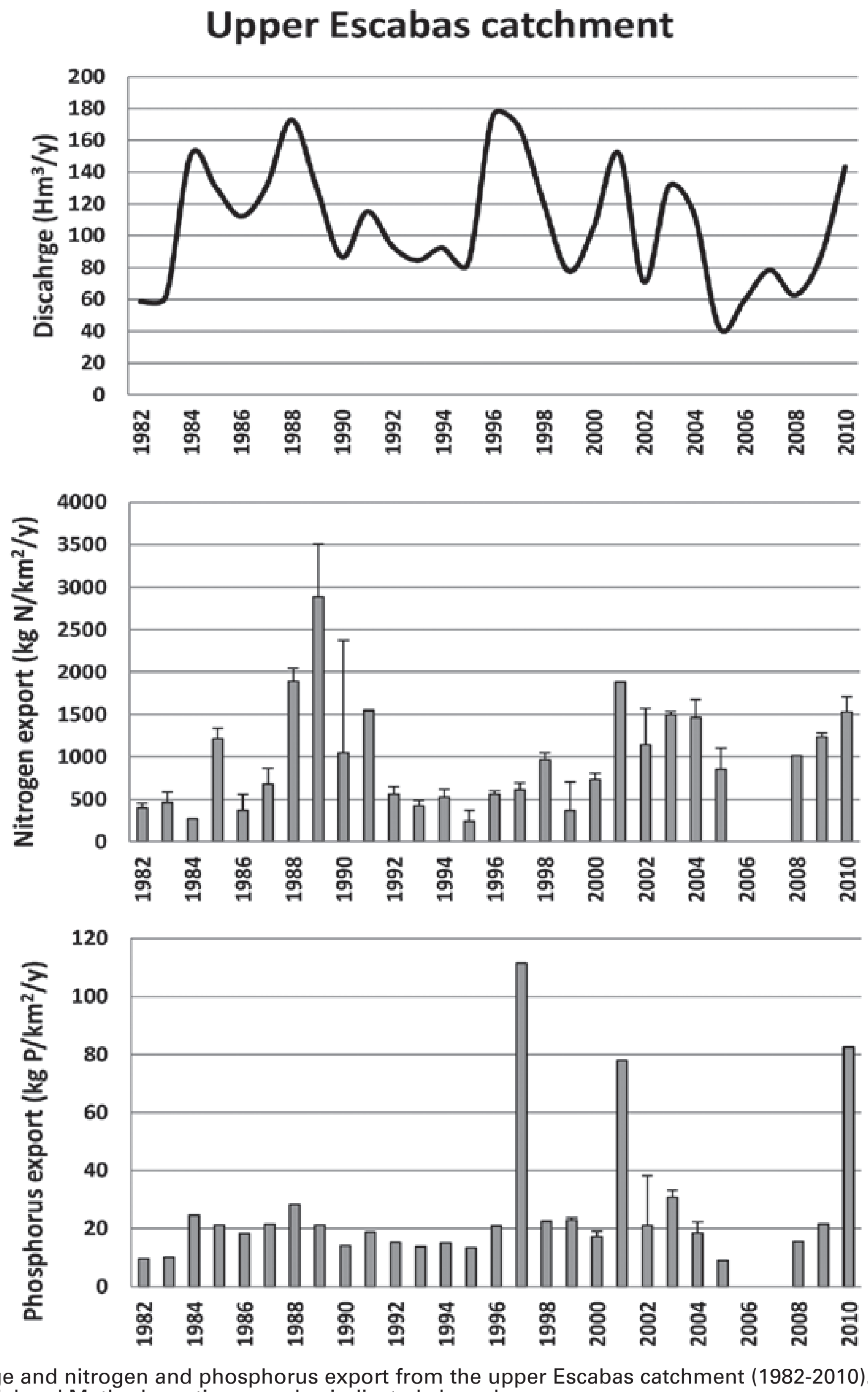

Figure 3. Yearly discharge and nitrogen and phosphorus export from the upper Escabas catchment (1982-2010). Annual errors, estimated as reported in the Material and Methods section, are also indicated above bars.

***Figura 3.** Caudal anual y exportaciones de nitrógeno y fósforo por la cuenca del alto Escabas (1982-2010). Los errores anuales, estimados según se describe en la sección de Material y Métodos, figuran sobre las barras verticales.*

trient concentrations, a fact which has rarely been found (Mussolf *et al.*, 2015). Power functions, which are usually analysed when dealing with concentration-discharge relationships, are only statistically significant for NH_4 in the Tajo catchment, the remaining concentration variables not showing any statistically significant power relationship with discharge for any sub-catchment. Such unfitting to power functions is more common than previously thought and frequently occurs in coastal streams (Diamond and Cohen, 2018). Their causes can be several (inputs of wastewater, differential runoff, inputs of groundwa-

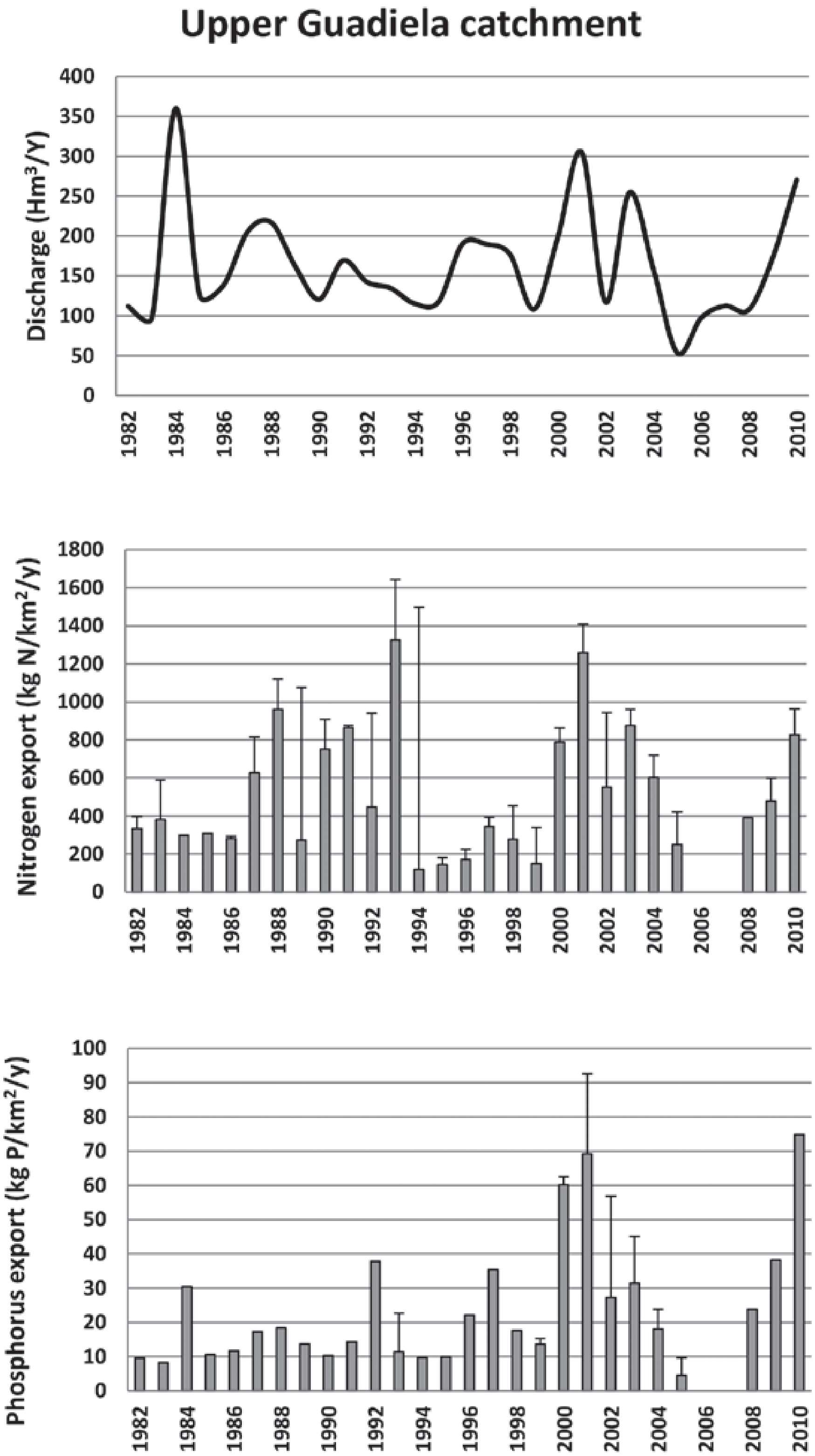

Figure 4. Yearly discharge and nitrogen and phosphorus export from the upper Guadiela catchment (1982-2010). Annual errors, estimated as reported in the Material and Methods section, are also indicated above bars.

Figura 4. *Caudal anual y exportaciones de nitrógeno y fósforo por la cuenca del alto Guadiela (1982-2010). Los errores anuales, estimados según se describe en la sección de Material y Métodos, figuran sobre las barras verticales.*

ter, changes in *in-situ* metabolism, sedimentation of particulate material, leakage towards the hyporheic zone...), but our surrogate of biological activity (% oxygen saturation) only rarely showed supersaturation suggestive of high in-stream photosynthesis. We have demonstrated that the lack of relationship between discharge and concentration also occurs in headwater sites, but we are unable to provide causal explanations for this at present. Therefore, most chemical species involved the studied Serranía de Cuenca sub-catchments behaved as chemostatic (Mussolf *et al.*, 2015), *i.e.* concentrations were invariant regarding discharge, a fact that could result from both catchment (including groundwater inputs) and in-stream processes counterbalancing discharge effects. As suggested above, photosynthesis was of minor importance in most sites and hence nutrient transformations are more prone to occur as a result of sedimentation and other physical processes, such as solute exchange between the stream channel and the underlying hyporheic zone.

Stagnant waters affect NH_4, NO_3, POC and DOC river concentrations and fluxes downstream in the Serranía de Cuenca by decreasing their variability. The same was true for SRP flux, but total P flux remained unaffected by water retention. As far as nitrogen was concerned the occurrence of wetlands at the local scale, which usually increase water retention in the catchment, has been shown to promote retention and N_2 and CH_4 outgassing (Pellerin *et al.*, 2004, Williams *et al.*, 2005), hence diminishing carbon and nitrogen export and its variability. As our study suggests, total P retention, however, does not appear to be enhanced by stagnant waters, as is usually reported (Bruland and Richardson, 2006), but the reason for this as yet remains unclear in the Serranía de Cuenca streams.

Catchment surface area and land use are unrelated with any nutrient flux, except cropland areas, which appear to control nitrate flux, albeit explaining less than a half of overall variability. The remaining fluxes do not seem to be controlled by any single measured factor, including forested areas. Export models of large catchments perform much better than models for small catchments (Smith *et al.*, 2005), and this could be the reason why we have not observed relationships of nutrient export and either catchment area or land use since our watersheds are small (Table 1).

All PCA analyses showed that most fluxes were represented by the first Factor (Table 7). However, the likely controlling variables of such a factor change. While they were salinity for the Upper Júcar catchments, it was land use for the Upper Tajo catchments (Table 7). So we dare to suggest that different sets of environmental variables might have controlled net fluxes in the different larger sub-catchments of Serranía de Cuenca. Other studies worldwide (Alvarez-Cobelas *et al.*, 2008, 2009, 2012) suggest that controlling factors of export are driven by the same variables at the regional scale, but not at the worldwide scale. However, here we have provided evidence that such controls can be different in nearby catchments sharing a great deal of climate and geological settings. Therefore, pristine catchments would appear to be very idiosyncratic as far as nutrient export is concerned.

Long-term nutrient export

Monthly rainfall partly explained one-month lagged monthly discharge in all three sub-catchments (R^2 = 0.24, 0.26 and 0.27, N > 400, $p < 0.05$, for the Cabriel, Guadiela and Escabas catchments, respectively). There were differences in long-term patterns of annual export between catchments. Whilst the N export from the two Tajo major sub-catchments (Guadiela and Escabas) showed a bimodal pattern, that of P was fluctuating throughout the study period (Figs. 3-4). In the Júcar basin, export from Cabriel river shows several peaks for both nutrients (Fig. 5). It is difficult to explain these differences if one takes the existing co-occurrence of discharge in the three catchments into account (see above). Discharge does not appear to be a predictor of concentrations for all yearly data of nutrients in the Guadiela and Cabriel sub-catchments, but it partly explains the N content in the Escabas sub-catchment. In other mountain environments rainfall-runoff appears to control nutrient export (Lee *et al.*, 2013), but this is not the case in the Serranía de Cuenca catchments whose behaviour might be more complex, probably being mediated by the limestone nature of the bedrock and its karstic hydrogeology (see the Study Site section), which results in high groundwater inputs to streams.

Nevertheless, only the P annual export is partly, albeit weakly, explained by discharge (and rainfall), but not by air temperature, in the long-term in the three studied sub-catchments, a fact that is consistent with the dominating surface transport of P from soils to streams (Haygarth *et al.*, 2005). If long-term prospects concerning rainfall and water availability in the Iberian Peninsula hold (Iglesias *et al.*, 2005), then it is likely that the P export would be reduced in pristine areas, but nothing can be said about the N export as yet. Annual N export has not shown any correlation with climatic features in our study, and this lack

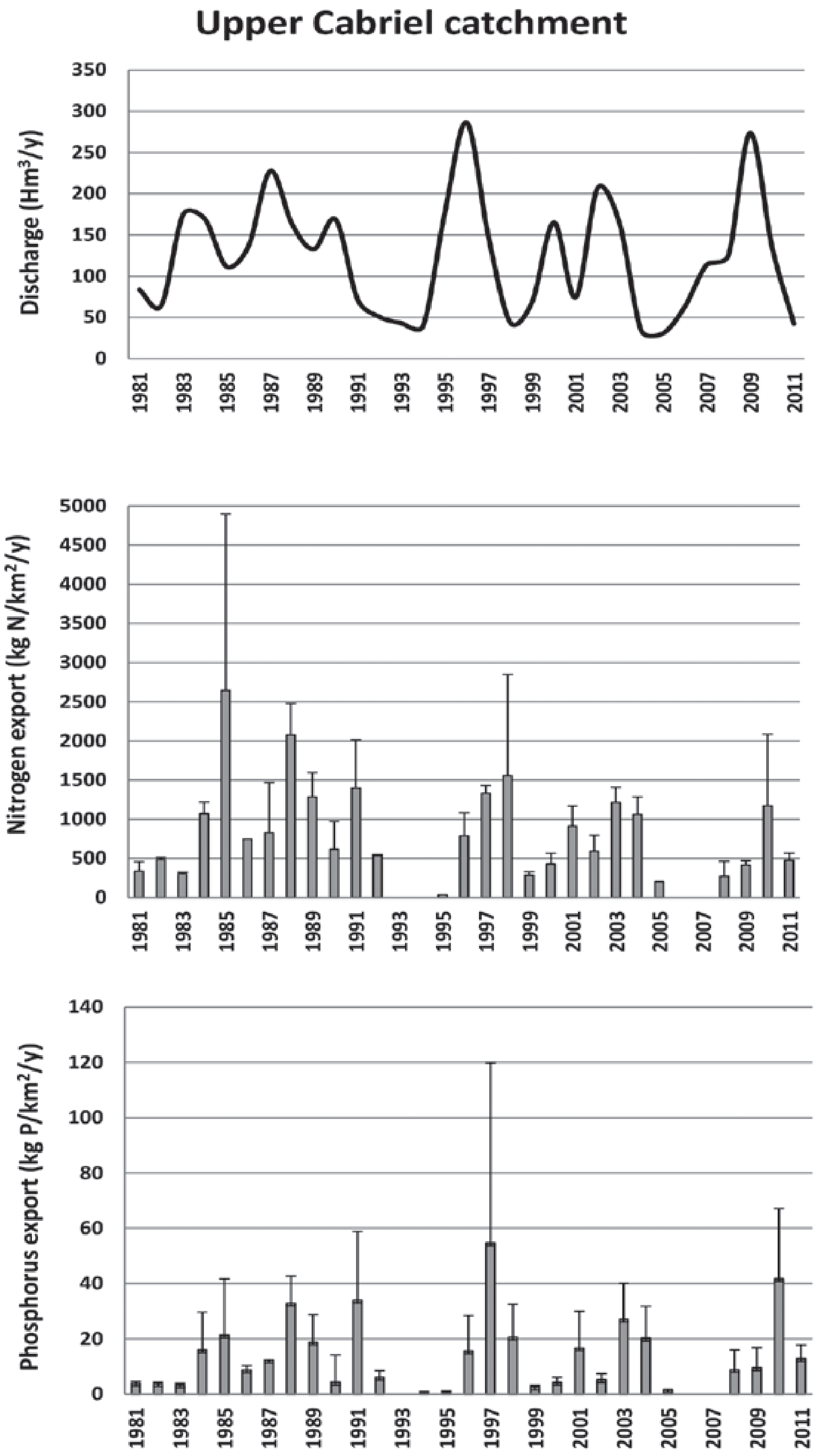

Figure 5. Yearly discharge and nitrogen and phosphorus export from the upper Cabriel catchment (1982-2010). Annual errors, estimated as reported in the Material and Methods section, are also indicated by above bars.

Figura 5. *Caudal anual y exportaciones de nitrógeno y fósforo por la cuenca del alto Cabriel (1982-2010). Los errores anuales, estimados según se describe en la sección de Material y Métodos, figuran sobre las barras verticales.*

of relationship might arise from the double pathway of nitrogen transport to streams, *i.e.* groundwater and surface (Mulholland and Hill, 1997) in a territory where water availability to streams might be strongly dependent upon groundwater discharge. It is also likely that the signal available is not enough to detect climate change effects on N export. A longer data set would certainly be necessary and we encourage increasing nutrient export studies in pristine areas because natural and/or global warming effects can be checked and even proved more easily there since other anthropogenic impacts (water scarcity due to excessive irrigation, water pollution and so forth) are negligible.

Concluding remarks

Our study has shown that (largely) pristine areas of streams behave in some unpredictable ways as far as nutrient concentrations and export are concerned. There have not been many studies on these topics recently, perhaps because governments are more interested in disclosing factors controlling water pollution in order to enhance its abatement. However, as our study has demonstrated, they deserve closer scrutiny because i) large fluctuations in nutrient concentration and export are the rule, thus implying that the variability of export fluxes is high despite their "pristine nature", ii) they cannot be ascribed to local man-made impacts, iii) whilst climate factors statistically explain part of the long-term variability, this share is not high, and iv) for nitrogen, long-term processes are not even explained by climate conditions. This conclusion, and the results from PCA analyses when we split the Tajo and Júcar catchments, suggest that there are different controlling factors of nutrient export in large, albeit close, basins which roughly share the same climatic and geological conditions.

Perhaps a smaller spatial scale of study could have performed better, but it would have needed more funding and sustained effort in the field and the lab. When dealing with nutrient export at the regional scale, such as ours, simple variables, easily recorded, are needed and hence variables at the coarser scale are preferred. However, these have not been enough to explain most of the variability involved.

We are well aware that increasing temporal resolution (*i.e.* more sampling over time) might have improved these results. It is obviously more expensive, and the fact is that we cannot afford them for the time being. Nevertheless, these results –whatever their preliminary nature is– will prove useful for checking future changes in pristine areas arising from climate change since the signal is unaffected by other man-made impacts at present (but obviously might be in the future). As such, these data could be useful as a baseline of nutrient export for future studies on the topic. Our results could also be interesting in their own right and be enough to promote further and more exhaustive hydrogeological studies (surface and groundwater ones as well) in pristine areas of the Iberian Peninsula, a topic that has been neglected in recent decades, maybe because of scarce research funding arising from the so-called "economic crisis".

Acknowledgements

La Perula hostel (El Tobar, Cuenca) proved to be a magnificent site and the owners were able to provide a base camp to undertake samplings easily across a relatively large, mountainous territory. José Luis Ayala and Alejandro Abadía are gratefully acknowledged for their skilful expertise which enabled the chemical analyses to be carried out very shortly after collection. The Júcar and Tajo Water Authorities supplied us with long-term discharge and chemical data of the upper Cabriel, Guadiela and Escabas rivers. We are also indebted to the Spanish Meteorological Agency (AEMET) for their long-term data on rainfall and air temperature. And last but not least, Raquel Morales (IGME) and an anonymous referee have given us very useful suggestions to improve an earlier draft of this study.

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