

Nitrogen exports at multiple-scales in a southern Chilean watershed (Patagonian Lakes district)

Christian Little · Doris Soto · Antonio Lara ·
Jaime G. Cuevas

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Abstract We evaluated nitrogen (N) export for various catchments in the San Pedro River watershed of South-central Chile (39°20' to 40°12'S) during the dry season (February to March). We measured concentrations and export of the various N species at 16 points from the Andean headwaters to the lowland portion of the watershed: eight main nested points along the main watershed and eight secondary points on tributaries. We expected that, given a downstream increase in pastureland and decrease in native pristine forest cover, inorganic forms of N (DIN) would increase downstream, while conversely, dissolved organic nitrogen (DON) would decrease compared with concentrations in the forested headwaters. Nitrogen concentrations did not show statistically significant differences among the nested

catchments. However, there were statistically significant differences in N concentrations associated with land cover among the tributaries. The results suggest that in the presence of base flow, natural landscape properties (barren land, lakes and rivers), explained most of the spatial variation in the N exports, while anthropogenic disturbance was not detectable. There was a negative relationship between DIN export and the coverage of lakes and rivers, suggesting that lakes might be acting as N traps. On the other hand, DIN, DON and total N exports were positively associated to barren land. Total nitrogen export during this 60-day dry season was less than 20 kg km⁻² and the annual export was not larger than 100 kg km⁻². This study documents the as yet pristine conditions of rivers in southern Chile.

C. Little (✉) · A. Lara
Instituto de Silvicultura, Universidad Austral de Chile,
Casilla 567, Valdivia, Chile
e-mail: clittle@uach.cl

C. Little · D. Soto · A. Lara · J. G. Cuevas
Núcleo Milenio FORECOS, Iniciativa Científica Milenio,
Valdivia, Chile

Present Address:
D. Soto
Fisheries and Aquaculture Department, FAO of UN,
Via delle Terme di Caracalla, 00100 Rome, Italy

J. G. Cuevas
Instituto de Investigaciones Agropecuarias
(INIA-Quilamapu), Chillán, Chile

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Introduction

Nitrogen (N) is one of the most studied elements in ecosystem ecology because it significantly affects productivity and eutrophication (Soto and Campos 1995; Vitousek et al. 1997). On a landscape scale, the N cycle has been appreciably altered due to anthropogenic activities (Galloway et al. 2003) making its understanding essential in areas with minimum human impact (Perakis and Hedin 2002). In rainy,

temperate regions N movement is closely connected to the hydrologic cycle (Likens and Bormann 1995; Perakis and Hedin 2001), where precipitation provides the main source, and surface runoff, subsurface and soil percolation supply the key form of transformation and loss (Likens and Bormann 1995; Vitousek et al. 1997).

Regarding the N cycle, the main difference between North America–Europe and South America is the absence of impact of acid rain in the latter region (Hedin et al. 1995; Weathers and Likens 1997; Montgomery and Buffington 1998; Galloway et al. 2003). Compared to streams in forests of the Northern Hemisphere, which have high inorganic nitrogen concentration (Likens and Bormann 1995; Compton et al. 2003), streams in pristine forests of the Southern Hemisphere have low concentrations of dissolved inorganic nitrogen (DIN) and high concentrations of dissolved organic nitrogen (DON) (Hedin et al. 1995; Perakis and Hedin 2002).

In native forest ecosystems in southern Chile, N load from wet deposition is low ($<1\text{--}5\text{ kg ha}^{-1}\text{ year}^{-1}$), and its export in the streams that drain these pristine forest areas is even lower (Hedin et al. 1995; Weathers and Likens 1997; Weathers et al. 2000; Perakis and Hedin 2001; Godoy et al. 2003). In contrast, in agricultural and livestock areas DIN is higher than DON because of intensive fertilizer use (Oyarzún et al. 2004; Oyarzún and Huber 2003).

The causes of difference in the N cycle between the temperate ecosystems in the Northern Hemisphere and the Southern Hemisphere are not clear. However, in the Southern Hemisphere there is a control of the N discharge that flows through fresh water systems provoked by a lower non-symbiotic fixation (low input of N to the system), and a lower N mineralization (lower nitrate generation), compared to Northern Hemisphere systems (Pérez et al. 1998, 2003).

Studies on a large spatial scale encompassing the same watershed are practically non-existent in Chile (e.g., Guevara et al. 2006) and uncommonly examined on the international literature (e.g., Biggs et al. 2004). Here we present results from a study of the San Pedro watershed, which is characterized by a mixed hydrological precipitation regime (rain and snow) and by a gradient of anthropogenic impacts (agriculture, livestock and silviculture) which increase as one moves downstream. Given the increase in pastureland cover and the decrease in

native forest cover downstream, inorganic forms of N should increase in magnitude; conversely, DON would decrease compared with forested headwaters. Thus, our objective was to determine the magnitude and composition of the N export in this gradient and to document changes in the DIN:DON ratio.

Study area

We selected the San Pedro River watershed ($39^{\circ}20'$ to $40^{\circ}12'$ S and $71^{\circ}40'$ to $73^{\circ}37'$ W), which drains $6,421\text{ km}^2$ in a westerly direction toward the Pacific Ocean transporting water from the Andean Mountain range to the lowlands including a portion of the Coastal Mountain range. The rivers cross the Intermediate Depression, which is a tectonic depression with a width of 50–60 km between the Andean and the Coastal Range.

The watershed encompasses two countries: a small upper section in Argentina (15% of the total watershed) and the majority in Chile. The drainage comes from a series of rivers that are chained to a series of glacial-formed lakes, which originate in the Lake Lacar catchment in Argentina and the Pellaifa Lake catchment in Chile. Both catchments join at Panguipulli Lake, which is connected by the Enco River to Lake Riniñue and San Pedro River downstream (Fig. 1).

A longitudinal transect (East to West) of near 170 km long (Figs. 1 and 2), was studied that included the main rivers and streams. The geomorphology of the watershed was shaped by glaciers, plate tectonics and volcanic activity (Laugenie 1982), with Andisol-type parent material following a gradient from young soils from the Andean range to developed soils from the Intermediate Depression, with distinct soil horizons (Tosso 1985). The climate is classified as a temperate rainy area with Mediterranean influence (Di Castri and Hajek 1976), presenting a gradual decline in precipitation from 4,000 to 5,000 mm over 500 masl in the Andean range, until 1,800–2,500 mm in the western portion of the watershed (Pezoa 2003).

The San Pedro River watershed maintains a diversity of ecosystems with land cover dominated by native forests at different successional states and disturbance regimes until 1550 (Lara et al. 1999). After that, the incursions and settlement of Europeans in Southern Chile introduced extensive forest fires

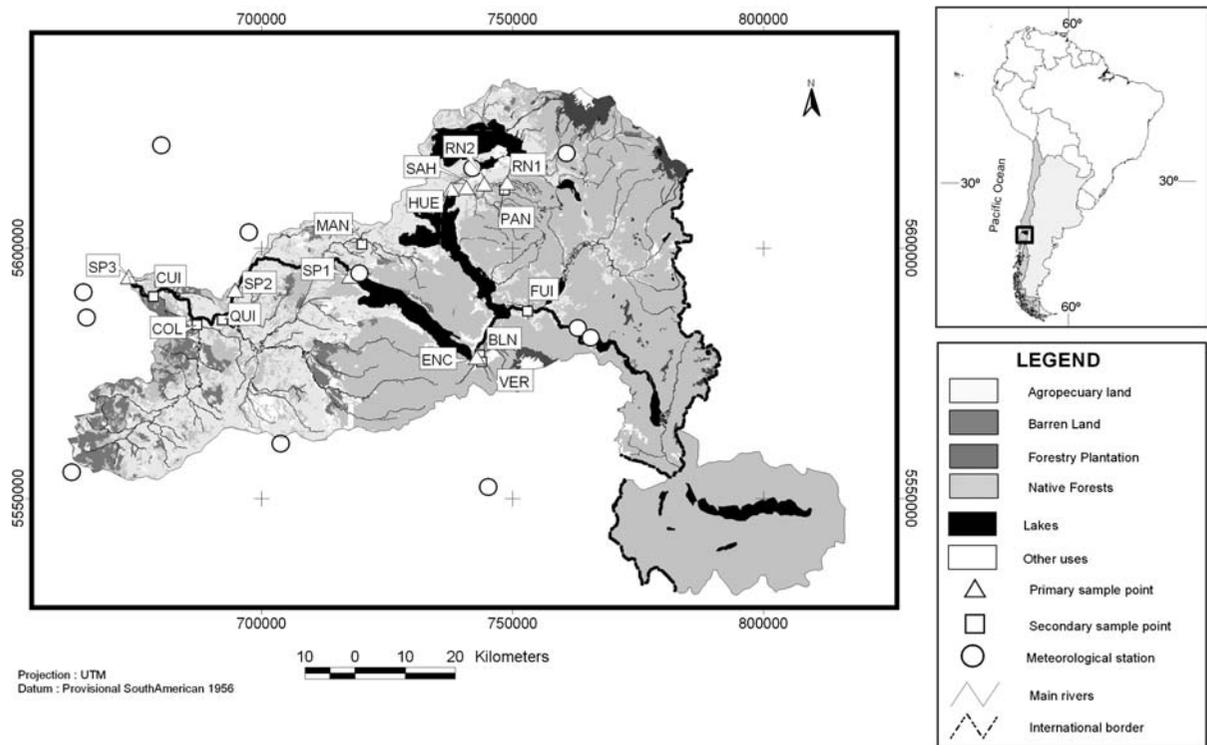


Fig. 1 Map of watershed locations indicating main and secondary points

and land clearing reducing the native forest cover. At present, in the Andean area, the forests are dominated by old-growth forests of *Nothofagus dombeyi*, *N. alpina*, *Laureliopsis philippiana* (mixed evergreen–deciduous) and *N. pumilio* (deciduous) forest types (Donoso 1981, 1993). At lower elevations in the Intermediate Depression the *N. obliqua*, *N. alpina*, *N. dombeyi* forest type (mixed evergreen–deciduous) dominates with a mosaic of pastureland and agriculture land. In the last three decades, these forests have been gradually replaced by fast growing exotic forest plantations, mainly in the Intermediate Depression and Coastal range. These plantations are dominated by *Pinus radiata*, *Eucalyptus globulus* and *E. nitens* (Lara et al. 2003, 2006).

Methods

Sampling design

In the San Pedro watershed, a total of 16 sampling sites were selected (Fig. 1). Eight of them were

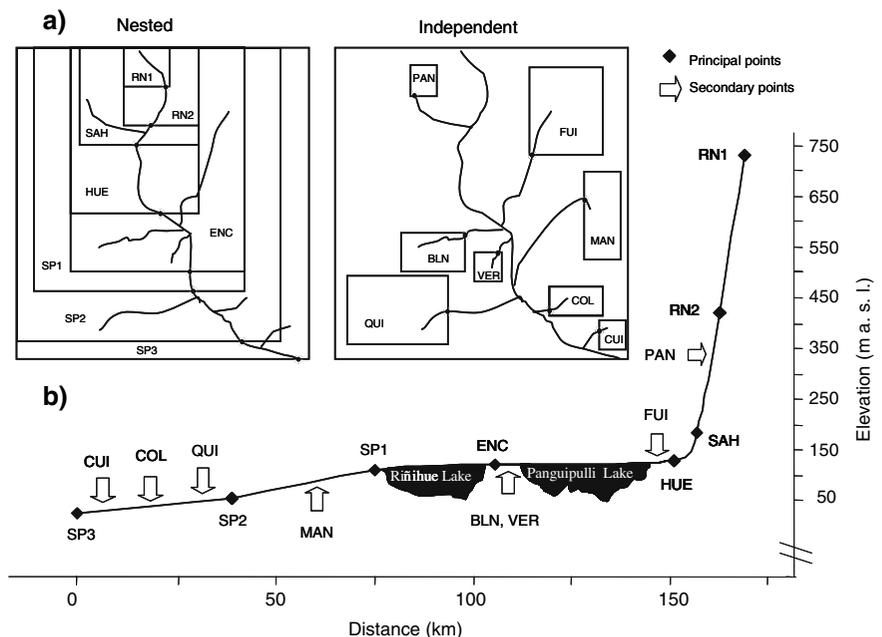
defined as “main points” located on what could be characterized as the main stem of the catchment (1st to 4th order streams). The catchment drainage area ranged from 17.1 to 5437.8 km² (Table 1). The remaining eight “secondary points” were located on tributary rivers and streams of varying orders located between the main points (Fig. 2a). In this case, the range of catchment area was 20.9–1312.2 km². The main points (Table 1) formed a series of nested catchments along the San Pedro River watershed. The catchments were located along an ecological and land cover gradient, representing the diversity of land cover types along the longitudinal transect in the San Pedro watershed (Fig. 1). The elevation range of sampling points was 45–730 masl (Fig. 2b).

Land cover classification was derived from the forest and land use 1:50,000-scale digital maps produced from 1:20,000 scale-aerial photographs and ground-truthing (CONAF et al. 1999). These maps were processed using Geographic Information Systems software (Arcview 3.3, Environmental Systems Research Institute, Inc., USA). The area of each catchment was determined from 1:50,000

Table 1 List of catchments, area and stream flow from February to March 2004

Abbreviation	Site river name	Catchment area (km ²)	Mean streamflow (m ³ s ⁻¹)
<i>Nested catchments/principal points</i>			
RN1	Ranguintulefu 1	17.1	0.54
RN2	Ranguintulefu 2	22.5	1.08
SAH	Sahuil	163.1	7.39
HUE	Huenaihue	949.5	33.75
ENC	Enco	2937.1	101.22
SP1	San Pedro 1	3281.1	121.50
SP2	San Pedro 2	3563.7	122.47
SP3	San Pedro 3	5437.8	143.86
<i>Independent catchments/secondary points</i>			
PAN	Panco	20.9	0.54
FUI	Fui	1312.2	33.32
BLN	Blanco	34.0	2.91
VER	Verde	24.1	1.25
MAN	Mañío	63.1	0.97
QUI	Quinchilca	910.9	14.2
COL	Collilefu	690.6	7.19
CUI	Cuicuilifu	90.6	2.16

Fig. 2 (a) Diagram of study area for nested and independent catchments. (b) Altitude gradient for main and secondary points



topographic maps with 25 or 50-m contour lines on digital format available from CONAF et al. (1999).

We used the following land cover categories: (1) old-growth forests, usually multi-species of heterogeneous structure regarding age, diameter, stratification and canopy cover; (2) young secondary

forests, originating from natural or human disturbances, of high production value under forest management schemes; (3) agriculture and livestock lands that replaced old-growth and secondary native forests; (4) exotic plantations of *P. radiata* and *Eucalyptus* spp. that have replaced natural forests in

the last 30 years; (5) lakes and rivers; (6) barren land, mainly areas above the treeline, rock outcrops and unvegetated slopes of volcanoes and (7) urban small towns and other rural settlements.

Water chemistry

One water sample was collected every 15 days at all sampling sites during February and March of 2004, with a total of four samples per site. These months have the lowest amount and variation in streamflow (Fig. 3). Samples were collected in plastic containers previously washed with 3% hydrochloric acid (2N) and rinsed with distilled water. All the samples were immediately transported in coolers ($\sim 5^{\circ}\text{C}$) and frozen following the standard methods for the examination of water and wastewater defined by the American Public Health Association (APHA et al. 1995).

Samples were analyzed in the Laboratory of Limnology at Universidad Austral de Chile. Nitrite (N-NO_2^-) was determined with sulfanilamide solution (detection limit $0.03 \mu\text{g/l}$), nitrate (N-NO_3^-) with sodium silicate (detection limit $1.5 \mu\text{g/l}$), ammonium (N-NH_4^+) with blue indo phenol (detection limit $0.8 \mu\text{g/l}$) and organic N with the Kjeldahl method ($1\text{--}1,000 \mu\text{g/l}$) (Zahradnik 1981). The diverse N forms are related in the following formulas:

- $\text{N}_{\text{total}} = \text{TN} = \text{N-NO}_2^- + \text{N-NO}_3^- + \text{N-Kjeldahl unfiltered}$

- Dissolved Inorganic Nitrogen = DIN = $\text{N-NO}_2^- + \text{N-NO}_3^- + \text{N-NH}_4^+$
- Dissolved Organic Nitrogen = DON = $\text{N-Kjeldahl filtered} - \text{N-NH}_4^+$
- Particulate Organic Nitrogen = PON = $\text{N-Kjeldahl in unfiltered water} - \text{N-Kjeldahl filtered}$
- Total organic nitrogen = PON + DON = $\text{N-Kjeldahl in unfiltered water} - \text{N-NH}_4^+$

Streamflow

Precipitation and temporal fluctuations in runoff and nutrient flux can significantly affect nutrient exports and their interpretation (Hood et al. 2003; McClain et al. 2003; Strayer et al. 2003; Cuevas et al. 2006). Thus, we concentrated the study during the dry period (February and March), which would emphasize the effects of land cover on nitrogen export, preventing the effects caused by high precipitation and streamflow variations during the rest of the seasons.

Since the rivers being sampled were of varying orders, different methodologies were used to measure streamflow. The main points RN1, RN2, SAH and the secondary points PAN, BLN, VER, MAN, QUI and CUI, all 2nd and 3rd order rivers, were measured with an Eijelkamp current meter and surveyed cross-section. Streamflow from SP1 and SP3, 4th order rivers and the secondary point COL, 3rd order river, were obtained from the discharge curves recorded by the Chilean Water Administration (Dirección General de Aguas, del Ministerio de Obras Públicas de Chile). The main points HUE, ENC and the secondary point FUI, all 4th order rivers, were calculated indirectly from regression curves between the streamflows of those rivers and that of SP1. This was necessary because all sites had gaps in the records for the last years, except for SP1. The runoff for SP2 was determined by the sum of the runoff from SP1 and the instantaneous river flow in the main tributary (MAN) between both.

All streams were measured at the same time of water samples collection for chemical analysis (that is, every 15 days in the same period of study). To determine the accumulated runoff over the study period, we used the mean instantaneous river flow measurement multiplied by the 60-day period (February to March). We observed a gradual decline in streamflow through the period, with small fluctuations (Fig. 3). Finally, the

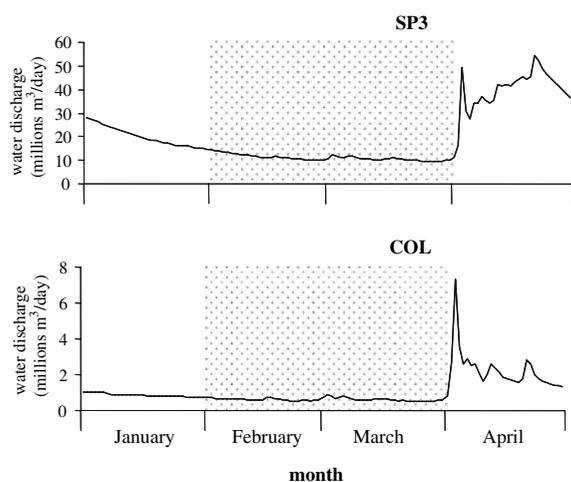


Fig. 3 Daily discharge for the main SP3 and secondary COL points

precipitation was recorded daily at 12 meteorological stations that geographically covered the area of the watershed (Fig. 1).

Nitrogen export

Strayer et al. (2003) and Caraco et al. (2003) stated that N export ($\text{kg km}^{-2} \text{time}^{-1}$) is one of the key variables for comparison in catchment studies, and the relationship of DIN to DON can act as additional ecosystem N status (Hood et al. 2003).

The N flux (kg day^{-1}) divided by the area of the watershed in squared kilometers provides the corresponding export value ($\text{kg km}^{-2} \text{day}^{-1}$). The N fluxes were calculated as the instantaneous measured concentration of the different N forms, including nitrate, ammonium, DIN, DON and PON, multiplied by the instantaneous streamflow at every moment of sampling. Therefore, we averaged the four values.

Data analysis

We used a one-way Analysis of Variance to determine whether the different N forms varied in magnitude respect to the concentrations and exports among sampling sites. Forward Step-by-step Multiple Regression analysis was utilized to establish the relationships between the export and seven categories of land cover. Previously, we checked for multicollinearity of land cover percentages, calculating Pearson correlations between these independent variables. Then, we developed a family of equations for the same dependent variable (e.g., DIN export), each equation including variables not mutually correlated. Finally, we selected the equation that achieved the maximum adjusted coefficient of multiple determination ($R^2_{\text{adj.}}$), with a significant model ($P < 0.05$) (Sokal and Rohlf 1995). All equations were checked for normal distribution of raw residuals, not requiring data transformation. The analyses were done with Statistica 6.0 software (Statsoft, Inc., Tulsa, OK, USA).

In the nested catchments we assumed independence because of the differences regarding precipitation, land cover, soil types, etc. We performed the Durbin Watson test to check for autocorrelation between Nitrogen exports in these catchments, and found independence.

Results

Land cover

In general, the proportion of old-growth native forest decreases from East to West, which is reflected in the observed proportions for the nested and independent catchments (Figs. 1 and 4). At the same time, the percentage of fertilized agriculture land (*Brassica napus*, *Solanum tuberosum*, *Triticum aestivum* and others) and livestock land (mainly *Trifolium repens* and *Lolium* spp.) increased up to 28.4% (Fig. 4a). The secondary forest percentage increased up to 13.2%. The exotic plantations, which are also fertilized, slightly increased their proportions with distance downstream, encompassing a small portion of the total study area. This is seen in SP1, SP2 and SP3. The barren lands increased downstream because when the watershed area increases, the high elevation area above treeline also increases (Fig. 4a).

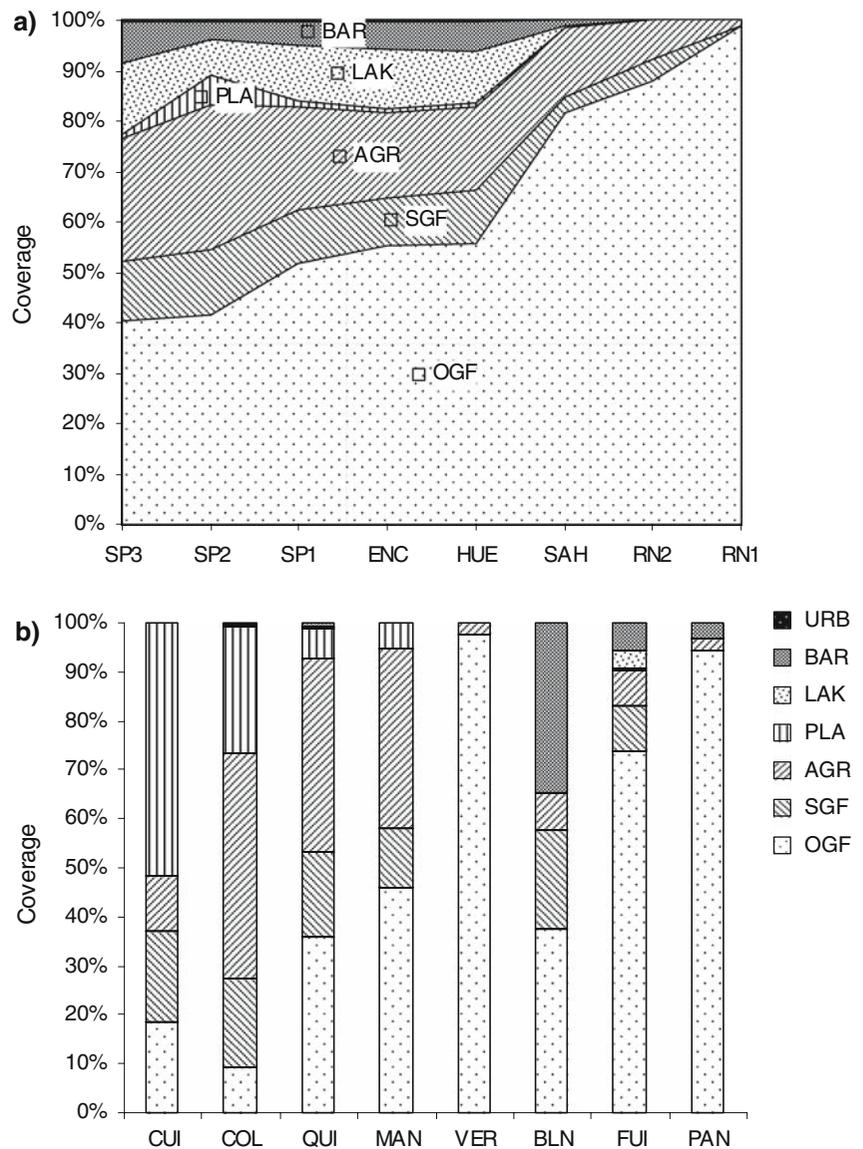
Hydrology

During the 60-day study period, the observed precipitation fluctuated between 250 mm in the East to 120 mm in the West of the watershed. This precipitation was 11% less than the mean observed precipitation during the last 30 years during the same months, and represented only 5% of the annual precipitation. These precipitation amounts were not capable of influencing the streamflow during the study period. Furthermore, based on historical records from the last 40 years at one monitoring station (SP1), the observed water flow at the end of the sampling period can be interpreted as base flow (Fig. 3). For both the main (nested catchments) and secondary points (independent catchments), there was a strong correlation between mean streamflow during February and March, and drainage area ($R^2 = 0.96$ and 0.97, respectively).

Nitrogen concentration

Total Nitrogen (TN) values in the main sampling points were over $50 \mu\text{g l}^{-1}$, more than 70% of which corresponded to organic N (Table 2). DON varied from 20.3 to $59.6 \mu\text{g l}^{-1}$ and was the dominant N

Fig. 4 Land cover in the nested (a) and independent (b) catchments. URB, urban area; BAR, barren lands; LAK, lakes and rivers; PLA, exotic tree plantations; AGR, agropecuary lands; SGF, second-growth native forests; OGF, old-growth native forests



form: concentrations increased from RN1 to HUE and then decreased from there to SP3 (Table 2). DON, DIN and PON did not show significant differences between sampling points ($F_{7,24} = 1.2$; $P = 0.3389$; $F_{7,24} = 0.36$; $P = 0.8942$; $F_{7,24} = 1.83$, $P = 0.1257$, respectively). Nitrate represented more than 70% of the DIN concentration (Table 2). Nitrate values for the main points were not statistically different from each other ($F_{7,24} = 1.03$, $P = 0.4389$).

For the secondary points, while organic N usually dominated TN, FUI and COL had DIN values over

50% of TN. Nitrate was again the main form of N in DIN concentrations, representing on average more than 80% of the DIN. Unlike concentrations at the main points, DIN (10.94 to 66.15 $\mu\text{g l}^{-1}$) and nitrate (7.25 to 62.8 $\mu\text{g l}^{-1}$) concentrations showed statistically significant differences between the tributary catchments ($F_{7,24} = 21.08$, $P = 0.0000$ and $F_{7,24} = 27.02$, $P = 0.0000$, respectively, Table 2).

Observed concentrations for the main points had a marked influence in the DIN:DON ratio. None of the main points had a DIN:DON value over 1. In contrast, various secondary points had values three

Table 2 Mean concentrations for main and secondary points ($n = 4$)

Site	Streamflow ($10^6 \text{ m}^3 \text{ 60 day}^{-1}$)	N_{total} ($\mu\text{g l}^{-1}$)	NO_3^- ($\mu\text{g l}^{-1}$)	NH_4^+ ($\mu\text{g l}^{-1}$)	DIN ($\mu\text{g l}^{-1}$)	DON ($\mu\text{g l}^{-1}$)	PON ($\mu\text{g l}^{-1}$)
<i>Main points</i>							
RN1	2.8	57.4 (9.1)	14.3 (0.95)	2.55 (1.42)	16.87 (1.82)	20.3 (5.63)	20.2 (3.91)
RN2	6.61	56.7 (10.5)	11.3 (1.75)	2.18 (0.69)	13.49 (2.18)	29.3 (10.6)	13.9 (4.32)
SAH	38.3	52.3 (19.6)	10.8 (0.95)	3.15 (2.19)	13.95 (3.11)	34.6 (17.7)	3.73 (0.68)
HUE	175.1	89.1 (8.94)	10.5 (0.91)	5.80 (0.46)	16.42 (0.77)	59.6 (7.71)	13.1 (5.46)
ENC	524.8	57.6 (4.40)	11.5 (2.68)	2.68 (0.75)	14.22 (2.72)	30.3 (3.99)	13.1 (3.78)
SP1	629.9	76.7 (21.9)	8.75 (1.25)	8.12 (2.70)	16.91 (2.04)	42.6 (17.8)	17.2 (2.71)
SP2	634.9	60.0 (19.4)	8.25 (3.20)	4.55 (1.30)	12.85 (4.09)	27.7 (12.2)	19.4 (5.99)
SP3	675.8	64.2 (15.3)	12.0 (1.70)	5.15 (2.65)	17.21 (4.28)	21.5 (9.41)	25.5 (7.74)
ANOVA		$F = 0.70$ $P = 0.6731$	$F = 1.03$ $P = 0.4389$	$F = 1.39$ $P = 0.2560$	$F = 0.36$ $P = 0.8942$	$F = 1.20$ $P = 0.3389$	$F = 1.83$ $P = 0.1257$
<i>Secondary points</i>							
PAN	2.84	45.0 (5.10)	10.3 (2.2)	2.68 (0.7)	12.99 (2.08)	13.8 (3.03)	18.3 (4.54)
FUI	183.1	47.0 (7.15)	19.8 (3.7)	7.03 (2.9)	26.84 (5.45)	7.99 (2.15)	12.2 (5.57)
BLN	15.1	50.2 (3.56)	7.25 (2.1)	3.65 (1.54)	10.94 (3.05)	26.2 (6.12)	13.1 (7.93)
VER	6.49	44.2 (7.83)	10.0 (1.9)	1.05 (0.14)	11.11 (1.99)	12.1 (1.98)	20.9 (9.02)
MAN	5.04	63.7 (7.29)	18.8 (3.1)	3.05 (0.43)	22.03 (3.27)	21.3 (6.87)	20.5 (7.66)
QUI	73.6	46.8 (11.0)	13.0 (3.3)	2.43 (0.94)	15.49 (3.12)	14.5 (6.61)	16.8 (2.76)
COL	37.3	108.5 (12.8)	62.8 (5.8)	2.9 (0.66)	66.15 (5.86)	24.1 (1.72)	18.1 (10.2)
CUI	11.2	61.8 (9.92)	20.8 (3.6)	4.55 (0.97)	25.43 (4.58)	22.7 (8.66)	13.7 (1.6)
ANOVA		$F = 3.52$ $P = 0.0003$	$F = 27.02$ $P = 0.0000$	$F = 1.75$ $P = 0.1444$	$F = 21.08$ $P = 0.0000$	$F = 1.54$ $P = 0.2012$	$F = 0.25$ $P = 0.9697$

The standard error is presented in parentheses (SE)

times greater (Fig. 5). We did not find a relationship with the coverage of agriculture and pasture land. The R^2 , $F_{1,6}$ and P values were: 0.0522, 0.3303 and 0.586, respectively, for the nested watersheds; 0.0469, 0.295 and 0.606, respectively, for the independent ones.

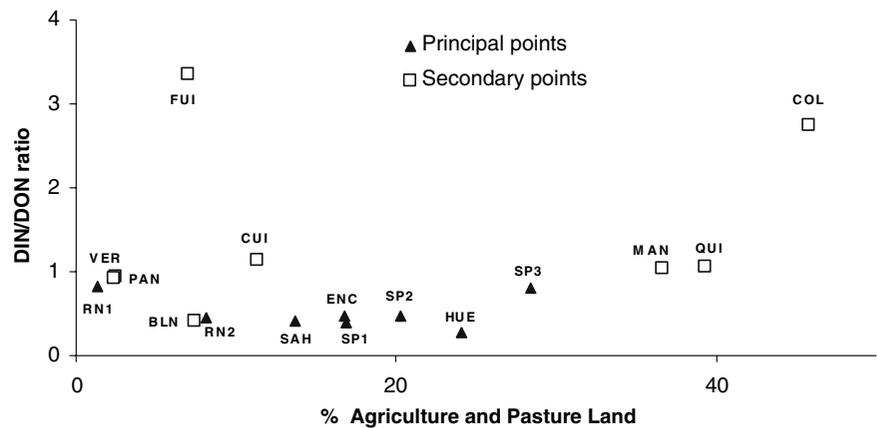
Nitrogen export

Nitrogen export in the nested catchments varied in the same way as N concentrations (Tables 2, 3), because concentrations were multiplied by a relatively constant ratio of streamflow to catchment area (0.026 to $0.048 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-2}$), with the majority of these values close to $0.03 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-2}$. Measured export values of TN ranged from 9.3 kg km^{-2} for SP3 to 16.6 kg km^{-2} for HUE (all values refer to a 60-day period). The Analysis of Variance indicated that there was no significant statistical difference in TN export for the main points ($F_{7,24} = 0.5843$ and

$P = 0.7618$). Export estimates in 60 days for organic N and inorganic N were not statistically significant among locations. DON export increased from 3.57 kg km^{-2} for RN1 to 11 kg km^{-2} for HUE and then a decrease to 3.2 kg km^{-2} for SP3. NO_3^- export differences were not statistically significant ($F_{7,24} = 1.1618$, $P = 0.3602$) but decreased from 2.31 kg km^{-2} in RN1 to 1.68 kg km^{-2} in SP3. TN export rates were dominated by organic N, with values of DON + PON near 75% of the total, where DON represented the greatest contribution with values often above 50%.

Exports of DON, PON and TN for the secondary points showed mean values smaller than the nested catchments (mainly due to lower concentrations) but with higher variability (Table 3). The runoff/catchment area ratio was more variable (0.01 to $0.086 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-2}$), thus the TN values ranged from 3.89 kg km^{-2} in QUI to 22.0 kg km^{-2} in BLN. In the secondary catchments the export differences in TN, DIN and

Fig. 5 DIN:DON ratios for the main and secondary sampling points in relation to the percent of catchment agricultural and pasture lands, as indicator of anthropogenic intervention



DON were statistically significant ($F_{7,24} = 22.536$, $P = 0.0000$; $F_{7,24} = 2.4830$, $P = 0.0452$; $F_{7,24} = 11.523$, $P = 0.0000$, respectively); but NO_3^- and PON were not ($F_{7,24} = 2.1227$, $P = 0.0801$; $F_{7,24} = 1.5136$, $P = 0.2101$, respectively).

Nitrate for the nested catchments, DIN, DON and TN for the independent catchments produced statistically significant regression models (Table 4). For the nested catchments, NO_3^- had a negative relationship with the percentage of exotic tree plantations, as well as that of lakes and rivers. The percentage of water bodies had a statistically significant beta-coefficient. In the independent catchments, the proportion of land cover occupied by barren land related positively with DIN, DON and N total and had statistically significant beta-coefficients. Bodies of water and agropecuary land related negatively with the DON, PON, and N total exports but no beta-coefficient was statistically significant.

Discussion

Magnitude of N exports and concentrations

The ecosystems in the Southern Hemisphere provide a unique opportunity to study the behavior of nutrient cycles. In contrast with those generally found in the Northern Hemisphere, where the nitrate exports are in average $870 \text{ kg km}^{-2} \text{ year}^{-1}$ (range 4 to 5,000; Strayer et al. 2003). N values in our study were comparatively low, with annual exports estimated in 100 kg km^{-2} for rivers that drain ecosystems with different levels of disturbance. Our values are within those reported by Hedin et al. (1995), Perakis and

Hedin (2001, 2002) and Godoy et al. (2003) for areas with low anthropogenic impact in Chile and Argentina where amounts range from 20 to 350 kg N km^{-2} .

In the Northern Hemisphere, DIN percentages are 83–98% with respect to total N (Lovett et al. 2000); in the ecosystems of Chile and Argentina this proportion is extremely low representing only 4% of the TN in streamwater (Perakis and Hedin 2002; Van Bremen 2002). According to Godoy et al. (2003), these ecosystems are operating in conditions comparable to pre-industrial times. Conversely, our results indicate that DIN represented a mean of 26 and 38% of TN concentration at the main and secondary points, respectively.

Trends in N concentration and exports

There were neither differences in N concentrations nor exports among the nested catchments. Conversely, various species of N varied among the independent catchments (Table 2). In the nested catchments, the results indicate low quantities of bio-available N (DIN:DON ratios lower than 1, Table 2, Fig. 5). Contrary to our expectations, we observed a decreasing trend in DIN:DON ratio with the increase in the anthropogenic influence in land cover toward agriculture and livestock (except for SP3), without a clear explanation (Fig. 5).

Different studies have shown concentration variations for different Nitrogen species in rivers resulting from changes in land cover (Oyarzún and Huber 2003; Oyarzún et al. 2004; Biggs et al. 2004; Cuevas et al. 2006). This effect is not clear in our study. Neither the regression models for nested nor

Table 3 Mean exports for main and secondary points ($\text{kg km}^{-2} 60 \text{ days}^{-1}$) ($n = 4$)

Export	RNI	RN2	SAH	HUE	ENC	SPI	SP2	SP3	ANOVA
<i>Main points/nested catchments</i>									
NO_3^-	2.31 (0.22)	2.84 (0.62)	2.50 (0.13)	1.93 (0.18)	2.01 (0.39)	1.67 (0.24)	1.59 (0.75)	1.68 (0.36)	$F = 1.1618$ $P = 0.3602$
DIN	2.68 (0.23)	3.35 (0.64)	3.20 (0.57)	3.03 (0.23)	2.49 (0.38)	3.33 (0.65)	2.45 (0.98)	2.46 (0.81)	$F = 0.4147$ $P = 0.8836$
DON	3.57 (1.23)	7.38 (2.51)	8.05 (4.13)	11.11 (1.85)	5.46 (0.81)	8.95 (4.44)	5.41 (2.85)	3.23 (1.68)	$F = 0.9897$ $P = 0.4618$
PON	3.51 (0.98)	3.19 (0.64)	0.89 (0.19)	2.41 (1.02)	2.39 (0.75)	3.39 (0.75)	3.56 (1.23)	3.57 (1.07)	$F = 1.1201$ $P = 0.3829$
N_{total}	9.77 (2.42)	13.91 (2.59)	12.15 (4.46)	16.55 (2.15)	10.35 (1.06)	15.67 (5.77)	11.42 (4.71)	9.26 (2.91)	$F = 0.5843$ $P = 0.7618$
<i>Secondary points/independent catchments</i>									
Export	PAN	FUI	BLN	VER	MAN	QUI	COL	CUI	ANOVA
NO_3^-	1.47 (0.51)	2.68 (0.46)	3.42 (1.26)	2.72 (0.62)	1.44 (0.20)	1.09 (0.32)	3.39 (0.42)	2.59 (0.55)	$F = 2.1227$ $P = 0.0801$
DIN	1.83 (0.57)	3.63 (0.69)	5.24 (1.82)	3.02 (0.65)	1.71 (0.24)	1.30 (0.32)	3.58 (0.46)	3.16 (0.68)	$F = 2.4830$ $P = 0.0452$
DON	1.87 (0.50)	1.16 (0.38)	11.42 (2.40)	3.31 (0.67)	1.80 (0.63)	1.21 (0.59)	1.33 (0.22)	2.66 (0.90)	$F = 11.5230$ $P = 0.0000$
PON	2.32 (0.51)	1.68 (0.76)	5.30 (3.08)	5.25 (2.03)	1.65 (0.66)	1.37 (0.27)	1.10 (0.70)	1.71 (0.29)	$F = 1.5136$ $P = 0.2101$
N_{total}	6.02 (1.03)	6.47 (0.88)	21.96 (1.69)	11.57 (1.50)	5.16 (0.94)	3.89 (1.07)	6.01 (1.24)	7.53 (1.28)	$F = 22.536$ $P = 0.0000$

The standard error is presented in parentheses (SE)

Table 4 Beta coefficients, adjusted coefficient of multiple determination ($R^2_{(adj.)}$), Fisher (F) and probability (P) values of the multiple regression models among the different nitrogenforms for export and the land cover categories in nested ($n = 8$) and independent catchments ($n = 8$)

Export (kg km^{-2} 60 days^{-1})	Old growth forest (%)	Second growth forest (%)	Agropecuary land (%)	Forest plantations (%)	Lakes and rivers (%)	Barren land (%)	Urban area (%)	$R^2_{(adj.)}$	F	P
<i>Nested catchments</i>										
NO_3^-	–	–	–	–0.37	–0.72*	–	–	0.72	$F_{2,5} = 9.88$	0.018
DIN	–	–	–	–0.50	–	–	–	0.13	$F_{1,6} = 2.05$	0.200
DON	–	–	–	–0.70	–	–	0.67	0.43	$F_{2,5} = 3.67$	0.100
PON	–	–	–	0.38	–	–	–	0.01	$F_{1,6} = 1.04$	0.350
N_{total}	–0.69	–	–	–0.81	–	–	–	0.25	$F_{2,5} = 2.14$	0.210
<i>Independent catchments</i>										
NO_3^-	–	–	–0.50	0.33	–	0.56	0.77	0.56	$F_{4,3} = 3.18$	0.184
DIN	–	–	–	–	–	0.73*	–	0.46	$F_{1,6} = 7.02$	0.038
DON	–	–	–0.13	–	–0.27	0.90*	–	0.94	$F_{3,4} = 35.66$	0.002
PON	0.57	–	–	–	–0.37	0.66	–	0.54	$F_{3,4} = 3.77$	0.116
N_{total}	–	–	–0.25	–	–0.23	0.81*	–	0.81	$F_{3,4} = 10.92$	0.021

* Parameters that turned out to be statistically significant in the model with $P < 0.05$

independent catchments showed trends that could be related to anthropogenic land covers. Instead, the natural components of the landscape, referred to lakes, rivers and barren areas, explained most of the Nitrogen export in the nested and in the independent catchments.

The previous results suggest that N loss is controlled by different variables that operate at different scales, where land cover change can vary in its ecological importance on the landscape. Further, some studies indicate that nutrient exports in a system can be related to other variables such as soil type, geomorphology and precipitation. Perakis and Hedin (2002) hypothesized that N export is related to the amount of recent precipitation. Cuevas et al. (2006), studying river catchments between 0.3 and 14.5 km² in southern Chile, found that when precipitation and geomorphology were added as predictors for concentrations, the fit of models improved, especially for nitrate, ammonium, phosphate and total phosphorus.

In the nested catchments there was a negative and significant relationship between nitrate and lakes and rivers cover, presumably because the lakes can act as traps. The decrease in Nitrate export between ENC and SP1 from 2.01 to 1.67 kg km^{–2} is probably related to the buffering effect of Riñihue Lake. From this reduction, we estimated a total retention of

420 kg of NO_3^- in the 60-day study period. Woelfl et al. (2003) have also suggested nitrogen retention by Lake Riñihue.

For the independent catchments, barren land explained the increase in the export of DIN, DON and N total, but the results are tightly linked, because all of them occur in BLN. This catchment is in the Andean Range, with large portions of barren land above tree line, covered by tephra characterized by a low capacity for water retention (Tosso 1985) and limited protection to leaking from rain. The large exports of DON in BLN could be associated with the large contents of N described for the soils in barren areas as humic and fulvic acids and humin by Borie et al. (2002). According to the results described by Weathers and Likens (1997) and Godoy et al. (2003), DIN exports may be explained by the atmospheric deposition that is not retained in the soil.

Conclusions

The main nested and secondary independent catchments provided an opportunity to study nitrogen concentrations and export with different approaches. The results suggest that in the presence of base flow, the properties of the natural landscape (barren land and land covered by lakes and rivers), explained most

of the spatial variation in the N exports, while anthropogenic disturbance was not detectable. These results differ from those reported for Northern Hemisphere ecosystems, which are exposed to atmospheric pollution.

In ecosystems with little or no exposure to atmospheric contamination which cover extensive areas in southern Chile, DIN:DON ratio can be an important indicator of the limitation of available N. A low DIN concentration restricts the primary productivity and keeps rivers and lakes in an oligotrophic state.

Our initial hypothesis stating that N exports and DIN:DON ratio would increase downstream along the anthropogenic disturbance gradient can be rejected. This may indicate the self-regulation capacity of the San Pedro River watershed. This new hypothesis needs to be tested by future studies, in order to prove the Nitrogen dynamics including the analysis of uptake, denitrification, dilution, as well as other possible explanations such as the influence of riparian vegetation along the river corridor, considering different spatial and temporal scales.

This study represents an addition to our understanding of temperate ecosystems in the relatively pristine environments of southern Chile and represents a baseline for future studies.

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