

Article

Watershed Dynamics in the Prespa Lakes: An Integrated Assessment of Stream Inflow Effects

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Abstract

The Prespa Lakes system, shared between Greece, the Republic of North Macedonia, and Albania, forms a significant transboundary, large-scale integrated freshwater ecosystem subject to multiple anthropogenic and natural pressures. This study focuses on the Greek part of the Prespa Lakes system with particular emphasis on the identification of the ecological and hydrological impacts of the contributing stream inflows on the lakes by examining the spatial variability in physicochemical and biological conditions and conducting water balance and isotopic analyses. Based on our results, streams draining into Lesser Prespa Lake exhibited more pronounced hydrological and physicochemical fluctuations than the Agios Germanos River connected to Great Prespa Lake, while ecological status classifications of all studied streams ranged from high to moderate. Furthermore, moderate ecological status conditions (mainly observed at the downstream stations) were closely associated with adjacent anthropogenic pressures, including agricultural drainage, livestock activities, irrigated croplands, and wastewater discharges. In addition, although both lakes were classified as mesotrophic, field data indicated greater transparency loss in Lesser Prespa than in Great Prespa Lake. Regarding the stream influences on Lesser Prespa Lake's water quality, nutrient loads induced changes in lake concentrations by roughly one month. Total nitrogen showed moderate stream–lake correlations ($R = 0.61$) and a strong negative correlation for total phosphorus ($R = -0.94$), suggesting substantial nutrient retention and processes within the lake. Water balance analysis revealed an annual water deficit for both Lesser and Great Prespa, with the latter exhibiting a markedly stronger and systematic long-term decline in water level. In the Lesser Prespa, seasonal fluctuations in water volume were primarily driven by excess rainfall, while stream inflows contributed minimally. Conversely, correlation analysis for Great Prespa identified surface inflow from the Ag. Germanos catchment as the dominant driver of water storage variability, surpassing direct rainfall, with strong correlations in both wet ($R = 0.79$) and dry ($R = 0.88$) periods. Isotopic compositions ($\delta^{18}\text{O}$, $\delta^2\text{H}$) did not differ significantly between the two lakes, indicating common recharge sources and strong evaporative imprints, while stream isotopic signatures highlighted spatial and seasonal variability in hydrological inputs. Seasonal and spatial variations were proved to be strongly influenced by both natural hydrological dynamics and anthropogenic pressures within the basin, while these findings reinforce the importance and the necessity of adopting holistic, cross-border management strategies that maintain the ecological integrity and the long-term sustainability of the Prespa Lakes ecosystem.



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1. Introduction

Natural lakes and artificial reservoirs, which together contain approximately 88% of the Earth's accessible surface freshwater, play a pivotal role in addressing the interconnected challenges of water, environmental, and climate sustainability. These lentic systems are fundamental to global water resource management and to enhancing climate resilience. They supply essential resources—including water, food, and energy—while mitigating the impacts of floods and droughts. Moreover, they sustain diverse ecosystems and biodiversity, thereby contributing substantially to human well-being, livelihoods, and economic development [1].

In terms of lakes' hydrology and water quality, climatic and weather factors—such as precipitation, temperature, wind patterns, and evaporation—play a crucial role in determining lake water levels, inflows, thermal stratification and nutrient cycling [2,3]. Additional influences include internal hydrodynamic processes regulating the distribution of oxygen, nutrients, and pollutants within the lake. Moreover, sediment resuspension and biological activity, including algal growth and organic matter decomposition, further impact water quality dynamics [4,5]. It is worth noting, though, that the quantification of internal nutrients in lakes is generally a challenging task. In addition, both the magnitude of internally derived nutrients and the processes governing their internal transport can differ substantially among lakes [6–8]. Even lakes characterized by comparable morphology, climatic conditions, and external nutrient loads may have internal circulation dynamics that vary substantially, as nutrients stored in sediments from past inputs can continue to influence water-column nutrient concentrations [9].

In the past, research and management efforts primarily concentrated on the internal dynamics of lakes and reservoirs, with an emphasis on limnological studies. Moreover, current ecological flow management practices still remain largely centered on individual water bodies—especially river systems—often overlooking the integrated needs of hydrologically connected and ecologically interdependent river–lake systems [10]. The World Meteorological Organization's (WMO) Global Water Resources Report underscores the increasing prevalence of drying basins and declining river discharges, which in turn reduce reservoir inflows [11]. Additional processes, such as the weakening or complete loss of river–lake hydrological connectivity, progressively disrupt the dynamic equilibrium and spatial distribution of water resources. As a result, these alterations are transforming once predictable, periodic hydrological regimes into increasingly unstable systems, thereby contributing to habitat fragmentation, biodiversity decline, and the deterioration of essential ecosystem services across major river basins worldwide [10,12]. In this direction, the WMO report calls for improved monitoring, enhanced data sharing, and strengthened transboundary cooperation, advocating for a policy transformation to better address the growing extremes of water surplus and scarcity [11]. Encouragingly, contemporary approaches have shifted toward integrated management of lakes in conjunction with their surrounding catchments, generating significant local and regional benefits while supporting multiple objectives such as water supply for domestic and agricultural use, hydropower generation, and the regulation of floods and droughts [1].

Integrated river–lake systems are essential resources for ecological sustainability and economic development [13,14]. River catchments serve as primary sources of water, sediments, nutrients (such as nitrogen and phosphorus) and potential contaminants, which are transported to downstream lakes. The magnitude and characteristics of these catchment-derived inputs are influenced by land use practices, including agriculture, industrial and urban development, which can contribute to water quality deterioration such as eutroph-

ication [4,15]. In turn, lakes regulate hydrological processes, freshwater storage, flow dynamics and support diverse ecosystems [3–5,16,17]. Numerous studies have emphasized the close interdependence between internal and external nutrient sources in lakes [18,19]. Net nutrient retention in lacustrine systems is governed by the interplay between sediment sequestration and internal recycling processes [20]. Nutrients originating from external inputs are gradually incorporated into sediments as particulate matter, algae, and detritus settle, while, simultaneously, internal cycling can remobilize nutrients from sediments back into the overlying water column. The relative contribution of these internal fluxes is strongly influenced by the magnitude of external loading [16,20,21]. Reductions in external nutrient inputs often induce a transition from turbid to clear water states, enhancing nutrient retention and enabling lakes to function as sinks for nutrients. Conversely, elevated external loading can increase turbidity and stimulate internal nutrient release, thereby amplifying nutrient fluxes and potentially destabilizing water quality [20]. Further relevant research efforts in the same direction have been conducted by [10,22,23]. Refs. [22,23] developed detailed water and nutrient budgets analyzing inflow/outflow patterns and seasonal and long-term trends in nutrient loads for all tributaries to Lake Okeechobee (Florida) and Lake Michigan, respectively. Finally, ref. [10] combined multi-stage field surveys with hydrodynamic simulations and fish community metrics to investigate the full-cycle ecohydrological impacts of a severe drought in the Yangtze River–Poyang Lake system (China). Therefore, and in accordance with the WMO, the effective management of such integrated and dynamic systems requires a thorough understanding of upstream–downstream linkages and the adoption of holistic approaches that account for watershed-scale dynamics, while ensuring the maintenance of water quality and ecosystem balance.

Based on the fact that even nowadays most of the relative research is still largely centered on individual water bodies (lakes or rivers) and to further reinforce the imperative for holistic river–lake management at the catchment scale, the present study investigates river–lake interactions within the transboundary Prespa Lakes ecosystem, shared by Greece, Albania, and the Republic of North Macedonia. The Prespa Lakes’ basin represents an ecosystem of outstanding international importance, globally recognized for its unique biodiversity [24]. It is also part of the Ohrid–Prespa Transboundary Biosphere Reserve, established under the auspices of UNESCO, and has been designated as a Ramsar Wetland of International Importance. Despite its significance, the hydrology and particularly hydrogeology of the basin are poorly investigated, partly because of its complexity, but, more importantly, because of its transboundary character [25], which poses substantial governance challenges and underscores the need for collaborative management approaches [26].

The present study focuses on the Greek part of the Prespa Lakes system while the whole comprehensive analysis is grounded in three core methodological pillars: (a) a water balance analysis aimed at evaluating the quantitative status of the examined water system; (b) an isotopic characterization, employed to verify the proportional contributions of different source waters to the Prespa Lakes system; and (c) in situ monitoring and sampling of hydrological, physicochemical and biological parameters to assess ecological status and support integrated system interpretation. Collectively, these methodological components allow for a comprehensive investigation of the interlinked hydrological and ecological processes governing the dynamics of the Prespa basin.

The water balance analysis serves as a fundamental tool for monitoring the quantitative status of water systems, particularly lakes. In light of the challenges posed by climate change and growing anthropogenic pressures, establishing a reliable water balance is essential for identifying the drivers of water level fluctuations in large lakes and unravelling complex interactions among them [27]. By accounting for all factors affecting the water budget, the estimation of water volume changes over time becomes feasible. This, in turn,

allows for the detection of long-term trends, providing a solid foundation for informed conservation strategies and policy decisions aimed at achieving sustainable water resource management at the basin level [28,29].

The stable isotope analysis of water (^{18}O and ^2H) constitutes the second methodological pillar, offering a versatile methodological tool for tracing hydrological processes in complex aquatic systems [30,31]. Isotopic techniques significantly enhance the investigation of hydrological processes, including the identification of water sources, the characterization of interactions among different water bodies, and the quantification of evaporation fluxes (e.g., [32–34]). Furthermore, the isotopic characterization of water inputs facilitates the application of advanced statistical approaches, such as Bayesian mixing models, to accurately quantify the proportional contributions of distinct water sources to aquatic systems, including rivers and lakes [35].

Finally, the in situ monitoring of hydrological, physicochemical, and biological parameters provides the ecological context necessary to link water quality with ecosystem health. In accordance with the Water Framework Directive (WFD), aquatic organisms (benthic macroinvertebrates, macrophytes, diatoms, phytoplankton and fish) are used as biological quality elements (BQEs) to assess the ecological quality status of European surface water bodies [36] in conjunction with other parameters. Diatoms and benthic invertebrates have long been used to assess anthropogenic pressures in rivers due to their sensitivity to different degrees of stress and at different time scales [37]. Diatoms can detect eutrophication gradients and have fast responses to environmental changes due to their small generation times (e.g., [38]). On the other hand, benthic invertebrates are more sensitive to organic pollution and general degradation, as well as to past stress events (e.g., [39,40]). The responses of the two groups to temporal changes also differ; macroinvertebrates generally depend on seasonality, whereas diatoms are independent of seasonality [37].

All in all, this research aims to elucidate the interconnectedness of water quality and ecological responses across this transboundary region, assessing the hydrological and ecological impacts of stream inflows on the Prespa Lakes, and providing a comprehensive understanding of the interactions among hydrological, ecological, and socio-environmental processes within this integrated river–lake system. By combining water balance analysis, isotopic data, and ecological indicators, the study seeks to strengthen the scientific foundation for sustainable water and ecosystem management, offering a robust framework for the long-term conservation of the unique transboundary Prespa Lakes ecosystem, while also contributing to the broader international discourse on integrated water resources management in complex transboundary systems.

2. Materials and Methods

2.1. Study Area

The study area is located in the south-eastern part of the closed Prespa basin, which hosts two homonymous lakes: Great Prespa Lake, situated in the central part of the basin, and Lesser Prespa Lake, in its south-eastern part. Northwest of the Prespa Lakes lies Lake Ohrid. The Prespa basin extends across the tri-national mountainous region at the north-western borders of Greece with Albania and the Republic of North Macedonia. It is characterized by a complex transboundary groundwater system that is hydraulically connected to both Lesser and Great Prespa Lakes as well as Lake Ohrid, while it exhibits a likewise complex geological and tectonic structure with formations belonging to the Sub-Pelagonian Zone (Greece), the West-Macedonian Zone (North Macedonia), and the Mirdita Zone (Albania) [41]. The Prespa region experiences a continental to sub-Mediterranean climate [42,43], situated within the transitional zone between Mediterranean and continental climatic regimes. It is characterized by warm, dry summers and cold, humid

winters. The average annual precipitation at the lake level is approximately 763 mm, while lake evaporation averages 833 mm for the period of 1951–2004; open-pan evaporation reaches about 1041 mm. In the surrounding mountains, however, annual precipitation likely exceeds 1200 mm [44]. The average annual air temperature in the study area at an altitude of 881 m, for the period 1961–1990, has been reported as 9.5 °C [45]. Monthly rainfall and air temperature data recorded at the Koula meteorological station, located in close proximity to the Prespa Lakes (Figure 1), for the study period are also provided in Table S1 (Supplementary File).

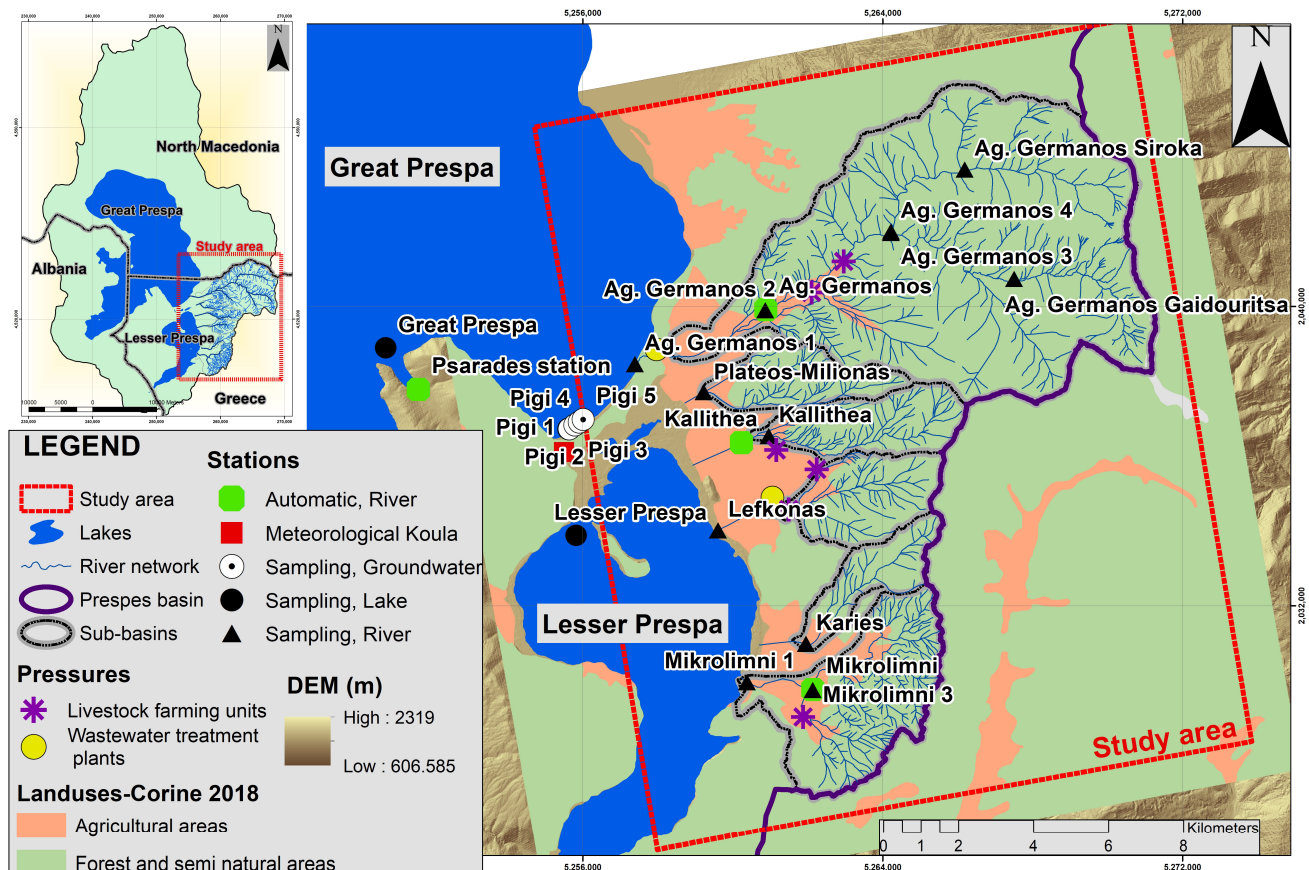


Figure 1. Map presenting the study area located in the south-eastern part of the closed Prespa basin, including all types of stations in Prespa Lakes and alongside the draining streams into them.

Lesser Prespa Lake belongs almost entirely to Greece, with the exception of a small southwestern section (approximately 11% of the total surface area) located in Albania. In contrast, Great Prespa Lake is shared among the three countries, with about 15% of its total surface area lying in Greece, 17% in Albania, and the remaining 68% in the Republic of North Macedonia. The surface area of Great Prespa Lake is 253.6 km² at an elevation of 846 m [46], whereas Lesser Prespa Lake covers 47.3 km² at an elevation of +850.60 m [47]. The surface extent of both lakes fluctuates over time, in response to variations in water level, which are in turn governed by the combined influence of meteorological, hydrological and anthropogenic factors.

The hydrographic network of the study area includes the Agios Germanos River, the only permanent river discharging into Great Prespa Lake, with an east–west flow direction. In addition, several intermittent or perennial streams discharge into Lesser Prespa Lake, including the Mikrolimni, Karies, Lefkonas, Kallithea, and Plateos–Milionas streams. The drainage basins of these streams and of the Agios Germanos River generally have an east/northeast–west orientation. The largest basin is that of the Agios Germanos River

(65.9 km²), followed by the Mikrolimni stream basin (15 km²), while the basins of the other streams range between 5 and 8 km² in area [48].

The most mountainous basin is that of the Agios Germanos River, with a maximum elevation of 2319 m, followed by the Kallithea stream basin, reaching up to 2109 m. In contrast, the Mikrolimni sub-basin is the most low-lying, with a mean elevation of 1056 m and a maximum elevation of 1496 m [48]. Overall, the sub-basins of the Agios Germanos, Plateos–Milionas, Kallithea, Lefkonas, Karies, and Mikrolimni watercourses are generally characterized by steep relief and similar geomorphological features, with the Agios Germanos sub-basin exhibiting the steepest slopes, while the Mikrolimni sub-basin displays the gentlest ones [49].

A key feature of the closed Prespa basin is its predominantly natural character. Among human activities, agriculture constitutes the primary source of pollution pressures in the study area, alongside intensive livestock units and wastewater treatment plants (Figure 1).

2.2. Sampling Network, Field Measurements and Laboratory Analyses

A systematic sampling approach was employed, involving multiple stations across key inflowing rivers/streams and Prespa Lakes. The sampling network was initially established in order to cover the Agios Germanos River spatially and then the main inflows to both Prespa Lakes, considering mainly the adjacent pollutant pressures and the hydro-morphological conditions of each stream. Field surveys were conducted in 2022–2023 at one station in each of the streams Karies, Lefkonas, Plateos–Milionas, Kallithea, Siroka (Agios Germanos 4), and Gaidouritsa (Agios Germanos 3). Two stations were established in the Mikrolimni stream (Mikrolimni 1, Mikrolimni 3) and in the main branch of the Agios Germanos River (Agios Germanos 1, Agios Germanos 2). During August–October 2023, two additional monitoring stations were added in the Siroka (Agios Germanos Siroka) and Gaidouritsa (Agios Germanos Gaidouritsa) streams, upstream of their confluence with the Agios Germanos River. In Lakes Lesser Prespa and Great Prespa, water sampling was carried out at one sampling station per lake on the same dates as those of the streams.

Monthly in situ discharge measurements were conducted at all stations by recording the water flow velocity and depth at various points across each riverbed.

Portable instrument (Hanna HI-98194 Multiparameter) was used to measure in situ water temperature, pH, electrical conductivity, dissolved oxygen concentration, total dissolved solids (T.D.S.), salinity, and turbidity while water samples were collected from the upper layer of the surface (5–10 cm) and transported to the hydrochemical laboratory of the Hellenic Centre for Marine Research (HCMR). In the laboratory, the water samples were filtered through 0.45 µm pore-size filters and subsequently analyzed for nutrients and chloride ions. Nitrate, nitrite, ammonium, and phosphate concentrations were determined using a Skalar continuous flow analyzer (CFA), following standard analytical methods. Specifically, ammonium was analyzed according to [50], phosphates following [51] and Standard Methods for the Examination of Water and Wastewater [52], as well as [53], while nitrate and nitrite determinations were performed according to [54].

Total nitrogen (TN) and total phosphorus (TP) were measured by wet chemical oxidation (WCO) [55,56], which converts organic N and P to inorganic nitrate and phosphate. Limits of quantification (LOQ) were 1 µg/L for nitrite, 2 µg/L for nitrate, 1 µg/L for phosphate, and 5 µg/L for ammonium. The estimation of measurement uncertainty for nutrient analyses is based on the implementation of internal Quality Control (QC) procedures and the routine analysis of Certified Reference Materials (CRMs). These measures allow continuous evaluation of analytical precision and accuracy, supporting the assessment of measurement uncertainty and ensuring the robustness of the reported data. The measurement uncertainty, estimated from internal QC results and CRM analyses, is within ±10% at

low concentration levels (close to LOQ) and within $\pm 5\%$ at higher concentrations for all nutrient determinations.

2.3. Ecological Status Assessment

2.3.1. Physicochemical Quality

To assess the physicochemical quality of the rivers/streams, the Nutrient Classification System (NCS) (Table S2 in the Supplementary File [57]) was applied, with modifications to incorporate the parameter of dissolved oxygen (DO) (Table S2 in the Supplementary File [58]). According to this method, the geometric mean (median) is computed for each sampling station and for each physicochemical parameter, while sampling stations are classified into one of five quality classes (High, Good, Moderate, Poor, Bad) based on the concentrations of nitrogen species (nitrate, nitrite, and ammonium) and phosphorus (orthophosphate ions).

Data were additionally elaborated based on the season sampled; concerning the wet period, a total of seven sampling campaigns were included, covering November–December 2022 and January, February, March, April, and May 2023. For the dry period, a total of five sampling campaigns were incorporated (June, August, September, and October 2023), with three campaigns specifically for the Plateos–Milionas, Agios Germanos Siroka, and Agios Germanos Gaidouritsa stations.

2.3.2. Biological Quality Elements

Benthic diatom samples were collected in spring (April) and summer (June) 2023 from submerged stones or aquatic vegetation in well-lit riffle zones and were processed in the laboratory with the hot hydrogen peroxide method, following European standards [59,60]. Cleaned frustules were mounted in Naphrax[®], and at least 400 valves were identified per sample to species level under a Nikon Eclipse 80i (Melville, NY, USA) phase-contrast microscope at 1000 $\times$ magnification, following the taxonomy of [61]. Biological status was assessed using the Specific Pollution Sensitivity Index (IPS- [62]) via OMNIDIA 5.3 software. IPS values were then divided by the IPS values of reference sites of RM1-type rivers and were, thus, converted to EQR (Ecological Quality Ratio).

Benthic macroinvertebrates were collected during two campaigns, in spring (April) and summer (June) 2023, with the semi-quantitative 3 min kick/sweep method plus a 1 min effort when bank vegetation existed, using a 250 mm $\times$ 300 mm D-shaped pond net (0.5 mm mesh size) (EN 27828:1994). During the 3 min sampling, all microhabitats were covered proportionally according to the matrix of possible river habitats [54]. Samples were preserved in 96% ethanol and identified to the family level. Biological quality was assessed using the Hellenic Evaluation System 2 (HESY2; [63]). The final EQR-HESY2 values were classified according to national thresholds for Mediterranean RM1 streams (for more details, see [63]).

2.3.3. Ecological Status

The ecological status of the rivers/streams was determined based on the “one-out, all-out” (OOAO) principle, applied in WFD classification, under which the lowest score of any individual quality element (in our case physicochemical and/or biological) defines the final classification.

Concerning the Prespa Lakes, the Denmark [64], the OECD [65] and the EPA [66], based on Carlson’s Trophic State Index—TSI [67] classification systems were utilized since they are among the most widely used indices for lake trophic status [68–70]. Based on these systems, lakes are classified into trophic levels (oligotrophic, mesotrophic, eutrophic, hyper-eutrophic) according to their content of various parameters such as Chlorophyll-*a*, nutrients and water transparency (Tables S3–S5 in the Supplementary Files).

2.3.4. Data Analysis

To investigate the effect of the measured environmental variables on biological quality, we applied linear models of the physicochemical variables measured in both sampling seasons (spring and summer) separately against the EQR values of the two biological groups (diatoms and macroinvertebrates). Statistical significance of the models was assessed using F-tests, with a significance threshold at $p < 0.05$. The physicochemical variables found to play a major role in driving biological quality in at least a biotic group were selected and, together with measured discharge, were used to assess stream stations' distance. All data were log-transformed for all analyses and normalized before calculating their Euclidean distance. For the linear models, R-studio v.2.5–6 (R Core Team [71]) (package 'stats') was used, and their visualization was done using packages 'ggplot2' [72] and 'ggpubr' [73]. Non-metric multidimensional scaling (NMDS) was applied to visualize the ordination of stations in reduced dimensional space [74], using PRIMER v7 [75]. NMDS solutions with stress values < 0.2 were considered acceptable.

To evaluate the influence of stream inputs on the Lesser Prespa Lake's nutrient dynamics, we initially calculated nutrient loads. Total phosphorus (TP) and total nitrogen (TN) loads in the lake were estimated by first determining the lake volume (L) from observed water levels on sampling dates. Measured nutrient concentrations were then multiplied by the corresponding volume to calculate loads. Streams' loads were calculated by multiplying the measured TP and TN concentrations at each station by the corresponding discharge. Then, correlations between nutrient loads of the inflowing streams and those of Lake Lesser Prespa were computed to evaluate their potential relationships.

2.4. Estimation of Water Balance Components for the Prespa Lakes

Key components of the lakes' water balance, namely direct precipitation, evaporation and surface inflows from the study area, were analyzed to understand their respective contributions to storage changes in Lesser and Great Prespa over time.

The water level for both lakes was recorded throughout the study period. For Great Prespa Lake, systematic water level observations were available via an automatic monitoring station located in Psarades station ($40^{\circ}49'48''$ N, $21^{\circ}01'40''$ E; Figure 1). In the case of Lesser Prespa Lake, measurements were carried out at Koula station ($40^{\circ}48'35.9''$ N, $21^{\circ}4'14.4''$ E; Figure 1) with a frequency of 2–4 days. Lake storage at the end of each month was specified based on observed water levels and the elevation–volume relationships for each lake, as established in previous studies [35,47]. Hence, monthly storage fluctuations were calculated by subtracting the storage volume of the previous month from that of the current month.

Direct rainfall over each lake was estimated employing precipitation data recorded at Koula meteorological station (Figure 1), operating under the coordination of the Society for the Protection of Prespa (SPP), after being aggregated on a monthly basis. Given its close proximity to the Prespa Lakes and its elevation at approximately +853 m, the station was considered representative for capturing the direct rainfall contribution. Monthly precipitation volumes were computed by multiplying the monthly rainfall depth by the corresponding median lake surface of each month, as derived from the elevation–area curves of the Prespa Lakes [35,47].

Evaporation losses from the lakes were estimated using the Penman method [76] and considering air temperature, solar radiation, wind speed and relative humidity data, recorded at Koula meteorological station. The resulting evaporation depth was translated into monthly volume losses by multiplying by the median lake surface area for each month.

Monthly in situ discharge measurements were available for Lefkona and Plateos–Milionas streams, thus enabling the estimation of their monthly surface outflow to the

Lesser Prespa Lake. For Ag. Germanos river and Mikrolimni and Kallithea streams, river discharge was retrieved by combining water level data from automatic monitoring stations (Figure 1) with site-specific stage–discharge rating curves established for these locations.

An isotope dataset ($\delta^{18}\text{O}$ and $\delta^2\text{H}$ of H_2O) previously reported in [35] was retrieved and incorporated in the present study to further support and refine the water balance model results. The referenced study analyzed the isotopic composition of lakes and inflowing streams within the Prespa system to quantify the relative contributions of different recharge sources (e.g., precipitation, tributaries). Isotope values were reported in per mil (‰) relative to Vienna Standard Mean Ocean Water (VSMOW). A detailed description of the isotopic sampling and analytical procedures is available in [35]. The analytical uncertainty reported was ± 0.03 for $\delta^{18}\text{O}$ and ± 0.1 for $\delta^2\text{H}$.

3. Results and Discussion

3.1. Ecological Status of Studied Streams

The ecological status of the studied streams exhibited seasonal variability between spring and summer. While some stations consistently showed high water quality, others experienced moderate quality degradation due to nutrient enrichment and occasional pollutant inflows. Ecological status of the studied streams ranged from High (Ag. Germanos 4, Ag. Germanos 3) to Good (Ag. Germanos 2, Kallithea stream) and Moderate (Ag. Germanos 1, Mikrolimni stream, and Lefkonas stream; Table 1).

Table 1. Streams' final ecological status indicating the downstream presence of pollutant pressures. Color coding depicts the different quality classes within the framework of the WFD (high-blue; good-green; moderate-yellow; poor-orange; bad-red).

Sampling Station	Physicochemical Quality	EQR IPS (Diatoms)	EQR HESY2 (Macroinvertebrates)	Final Ecological Status
Lefkonas	High	Good	Moderate	Moderate
Kallithea	High	Good	High	Good
Mikrolimni 1	Good	Moderate	Moderate	Moderate
Mikrolimni 3	High	Good	Moderate	Moderate
Ag. Germanos 1	High	Good	Moderate	Moderate
Ag. Germanos 2	High	Good	Good	Good
Ag. Germanos 3 (Gaidouritsa stream)	High	High	High	High
Ag. Germanos 4 (Siroka stream)	High	High	High	High

Station Agios Germanos 1 was affected by elevated concentrations of nitrites, nitrates, ammonium, and phosphates, particularly during September and October 2023 (Table S6 in the Supplementary File). Consequently, only two Dipteran families, Simuliidae and Limoniidae, were recorded during the summer period, both of which are relatively tolerant to pollution (Table S7 in the Supplementary File). Moreover, the low abundance of these families suggests the occurrence of one or more significant pollution events. Furthermore, the ecological status ranged from Good (wet season; Table S9 in the Supplementary File) to Poor (dry season; Table S10 in the Supplementary File).

Both stations of Mikrolimni stream exhibited moderate ecological status, concerning not only the wet (Table S9) and dry (Table S10) seasons but also the final status (Table 1; Figure 2), with persistently high nitrate and nitrite concentrations throughout the sampling

period, including February and March (Table S6 in the Supplementary File). In terms of diatom communities, genus *Sellaphora pupula*, known for its tolerance to pollution and elevated nutrient concentrations, dominated during summer. Macroinvertebrate-based quality assessments classified the stream as moderate (Table S7 in the Supplementary File), with dominance of pollution-tolerant families, such as Simuliidae, Chironomidae, Gammaridae, Planorbidae, and Baetidae. Near station Mikrolimni 3, both point-source pollution (livestock farming units) and diffuse pollution sources (cultivated lands surrounding both stations) were identified (Figure 2).

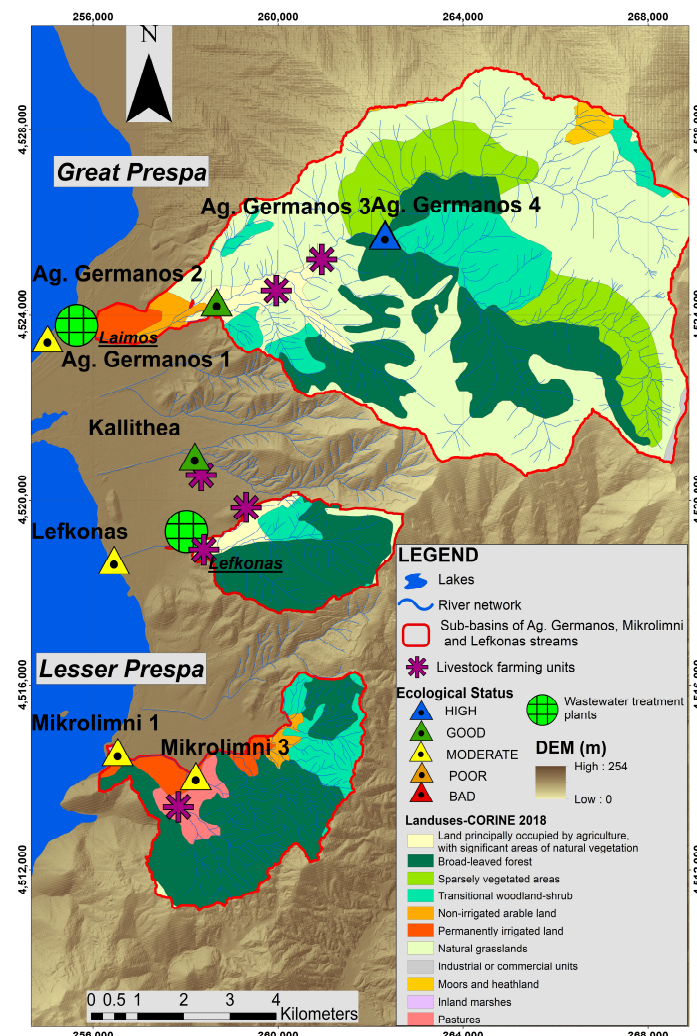


Figure 2. Map illustrating the ecological status of the streams discharging into the Great and Lesser Prespa Lakes, accompanied by pollution sources (livestock farming activities and wastewater treatment plants) and land uses observed at the sub-basins of streams with moderate ecological status (Agios Germanos Station 1, Mikrolimni, Lefkonas).

The moderate ecological status of the Lefkonas stream (Table 1; Figure 2) was attributed to elevated concentrations of nitrates, ammonium, phosphates, and total phosphorus, particularly during January, May, June, and August 2023 (Table S6 in the Supplementary File). At these increased concentrations, it is concluded that the effluent discharge from the Kallithea–Lefkonas wastewater treatment plant has contributed since the sampling station (Lefkonas stream) is located downstream of the plant’s outlet. Benthic sampling in the Lefkonas stream was conducted only in the spring due to water scarcity during the summer. The ecological status ranged from moderate (based on macroinvertebrates) to near the threshold between good and moderate (based on diatoms). In addition to

the wastewater treatment plant, other point-source pollution pressures upstream of the Lefkonas sampling site included livestock farming facilities, along with diffuse pollution sources such as agricultural land and pastures (Figure 2).

Based on the results, moderate ecological status was directly linked to adjacent pressures, including agricultural drainage ditches, livestock farming, irrigated croplands, and wastewater treatment plants.

Among the physicochemical variables measured, several had direct effects on the biological quality elements assessed. In particular, increases in conductivity and ammonium concentrations negatively affected diatom-based biological quality (LM conductivity: $F_{1,13} = 8.09$, $p < 0.05$; LM NH_4 : $F_{1,13} = 12.63$, $p < 0.01$), but showed no significant effect on macroinvertebrate-based quality (LM conductivity: $F_{1,13} = 1.75$, $p = 0.209$; LM NH_4 : $F_{1,13} = 4.04$, $p = 0.066$; Figure 3). The opposite pattern was observed for dissolved oxygen; higher dissolved oxygen levels improved macroinvertebrate quality (LM DO: $F_{1,13} = 6.83$, $p < 0.05$) but had no detectable effect on diatom quality (LM DO: $F_{1,13} = 0.01$, $p = 0.923$; Figure 3). Elevated phosphorus concentrations negatively affected the biological quality of both diatoms (LM PO_4 : $F_{1,13} = 11.71$, $p < 0.01$) and macroinvertebrates (LM PO_4 : $F_{1,13} = 4.89$, $p < 0.05$; Figure 3).

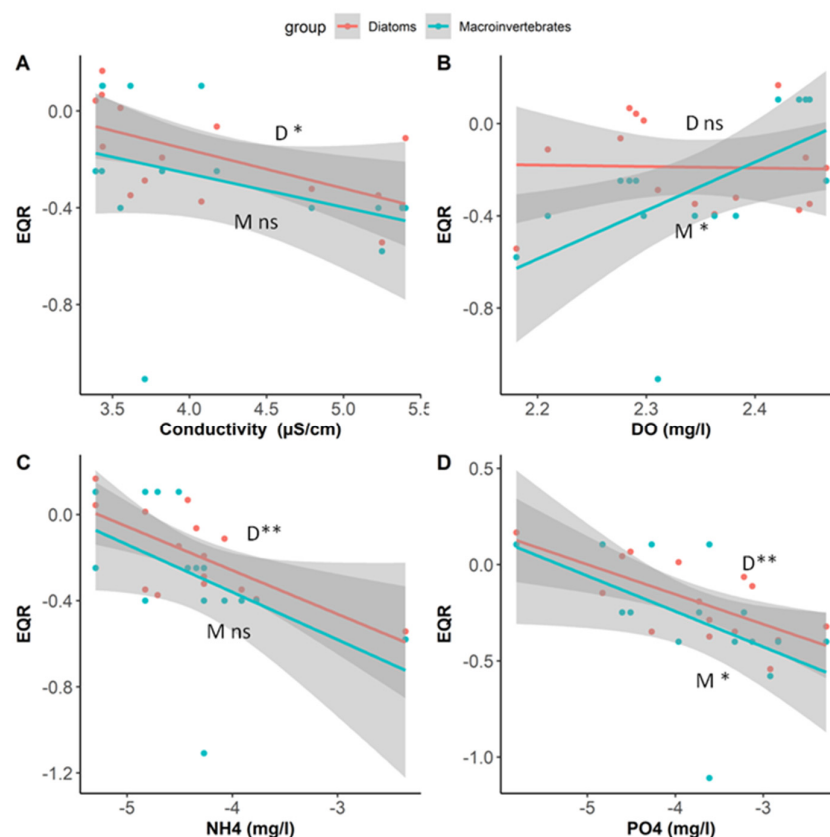


Figure 3. Effect of conductivity (A), dissolved oxygen (B), NH_4 (C) and PO_4 (D) on biological quality of the two studied biological quality elements (diatoms—D and macroinvertebrates—M). Data correspond to the two sampling campaigns for biota, during spring and summer 2003. Both y and x axes are in log scale. Significance of linear models is indicated as ns (non-significant), * p -value < 0.05 , ** p -value < 0.01 . Funnels represent 95% confidence intervals.

An evident grouping of the stream stations based on their connection to the Great Prespa Lake (Ag. Germanos stream) and the Lesser Prespa Lake (Kalihea, Lefkonas, Mikrolimni streams) was observed, which remained consistent across seasons (Figure 4). The environmental variables used were conductivity, dissolved oxygen, NH_4 , and PO_4 , and

all were shown to affect stream biological quality together with water discharge. Based on this observation, it can be assumed that streams and their associated quality exert different influences on the two Prespa Lakes.

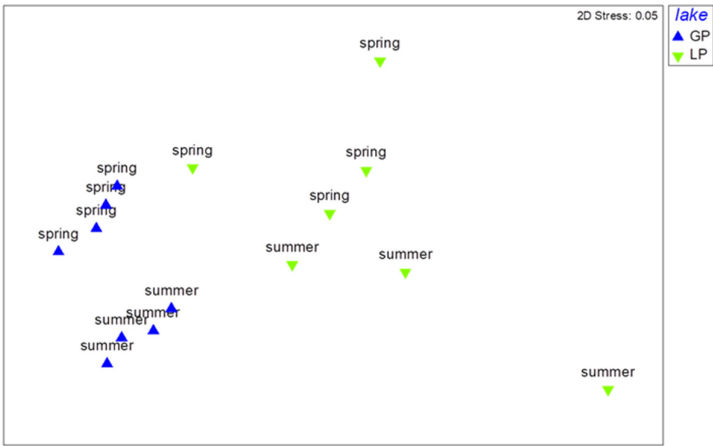


Figure 4. Non-metric multidimensional scaling (Euclidean distance) of the different stream stations based on physicochemical parameters that affect biological quality (conductivity, DO, NH₄, PO₄) and discharge. Data were log-transformed and normalized before the analysis. GP represents stream stations that are connected to the Great Prespa Lake, and LP stream stations that are connected to the Lesser Prespa Lake. The stress of the analysis equals 0.05, indicating an excellent representation of the data in reduced dimensions.

3.2. Water Quality and Trophic Status of Prespa Lakes

Based on median TN concentrations and the Denmark classification system [64], the trophic status of both depth layers in Great Prespa Lake was assessed as High (Table 2). Median TP concentrations indicated a High status for Lesser Prespa Lake and a Good status for both depths of Great Prespa Lake, whereas Secchi depth measurements classified water quality as Poor in Lesser Prespa and Moderate in Great Prespa Lakes, respectively.

Table 2. Classification of Prespa Lakes’ water quality/trophic status (Lesser Prespa N = 11, Great Prespa N = 12).

Station	Depth (m)	Median TN (mg/L)	Median TP (mg/L)	TSI Average	Denmark System (TN)	Denmark System (TP)	Denmark System (Secchi)	OECD (1982)-TP	OECD (1982)-Secchi	EPA (2000)
Lesser Prespa	0–1	0.44	0.01	44.94	High	High	Poor	Mesotrophic	Eutrophic	Mesotrophic
Great Prespa	0–1	0.401	0.0185	43.68	High	Good	Moderate	Mesotrophic	Mesotrophic	Mesotrophic
Great Prespa	15	0.372	0.021		High	Good		Mesotrophic		Mesotrophic

According to the OECD classification system [65], the mean TP concentration corresponded to mesotrophic conditions in both lakes, while Secchi depth values suggested eutrophic conditions in Lesser Prespa and mesotrophic conditions in Great Prespa.

Similarly, the EPA classification system [66] categorized both lakes (according to average values of TSI) as mesotrophic, based on TP concentrations and Secchi depth measurements (Table 2). The measurements considered for the calculation of the trophic status in the lakes were the sampling campaigns of November–December 2022 and January, February, March, April, May, June (2 measurements), September, and October 2023 (in total 11 measurements). In Lake Great Prespa, an additional water sampling was conducted in August 2023 (in total 12 measurements) at both depths.

In addition, the trophic status classification of the two study lakes, based on the OECD system [65] and Secchi depth measurements, is presented for the periods 1996–2014 [77] and 2015–2023 (Society for the Protection of Prespa—SPP) [78]. Overall, Lake Great Prespa has most frequently exhibited mesotrophic conditions, with episodic shifts to eutrophic

status in several years, whereas Lake Lesser Prespa has predominantly been classified as eutrophic, with occasional occurrences of hypertrophic conditions. This pattern indicates a more advanced degradation of water transparency in Lesser Prespa compared to Great Prespa, although both lakes display broadly similar interannual trends in Secchi depth values (Figure 5).

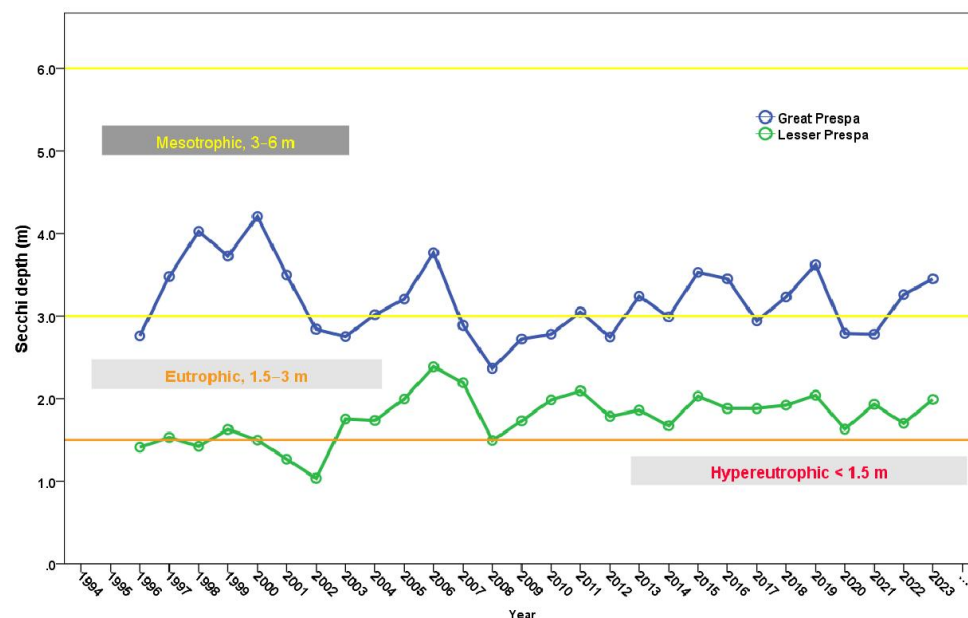


Figure 5. Annual Secchi depth (m) and associated OECD (1982) trophic status in the Prespa Lakes, indicating lower water transparency in Lesser Prespa (N = 28).

Complementary to this, it is reported that according to the River Basin Management Plans (RBMPs), the ecological status of Great Prespa Lake has fluctuated, being classified as Moderate (2014; [79]), Good (2017; [80]), and again Moderate (2024; [81]), while its chemical status has consistently been Good. Great Prespa is naturally nutrient-rich, with sediment N and P contents over the past 10,000 years and diatom concentrations indicating conditions between Good and Moderate ecological status. Over the last 1000 years, however, the lake has transitioned from a nitrogen-dominated to a phosphorus-dominated system, a shift that has intensified over the past 500 years. This anthropogenic influence has altered planktonic communities, favoring blooms of potentially toxic cyanobacteria, such as *Anabaena flos-aquae*, during summer and autumn.

RBMPs classified Lesser Prespa Lake as Poor, later as Moderate (2017; [80]), while it remained Moderate in 2024 [81]. Chemical status improved from below-Good to Good, while ecological status remained Moderate due to fish and zoobenthos communities and phosphorus concentrations indicative of mesotrophic conditions. Moreover, a previous study [82] indicated that pollution in the Prespa Lakes arises from both diffuse sources, such as agricultural nutrient runoff, and point sources, including domestic and industrial effluents and landfill leachates. These inputs contribute nutrients, hazardous compounds, and agricultural residues directly to the lake basins, influencing water quality and ecological status.

Effects of Stream Inflows on Lake Nutrient Dynamics and Retention

The Kallithea, Lefkonia, Plateos–Milionas, and Mikrolimni streams exerted a clear influence on nutrient dynamics in Lesser Prespa Lake, with TN and TP loads in the streams preceding lake responses by approximately one month. Peaks in stream-TN during December 2022 and February 2023 corresponded to elevated lake-TN in January and

March 2023, respectively, while declines in April–May 2023 were mirrored by reduced lake concentrations (Figure 6a). TP loads exhibited a similar, though less pronounced, temporal pattern. Stream nutrient fluctuations closely tracked variations in streamflow (Figure 6b), whereas lake nutrient concentrations were further modulated by changes in water volume and sedimentation processes.

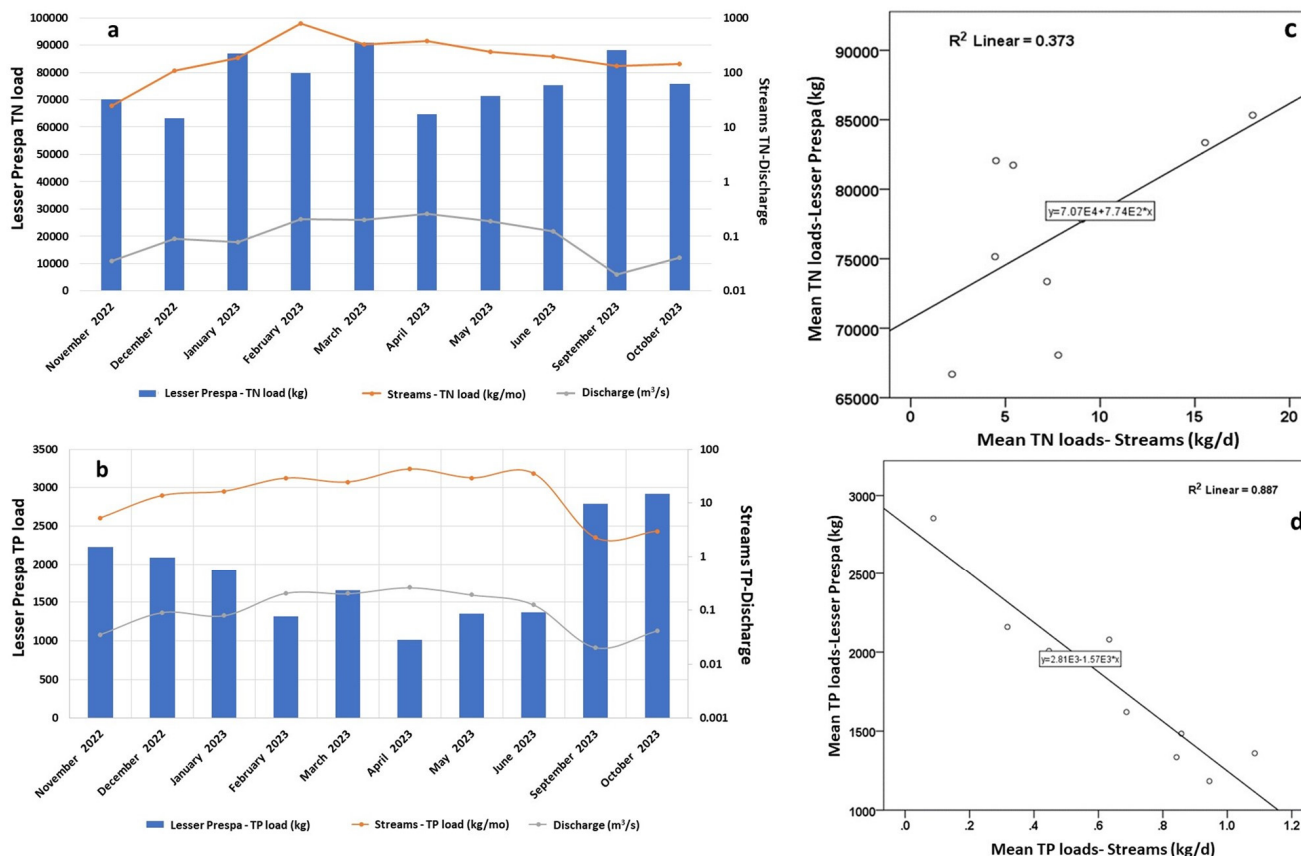


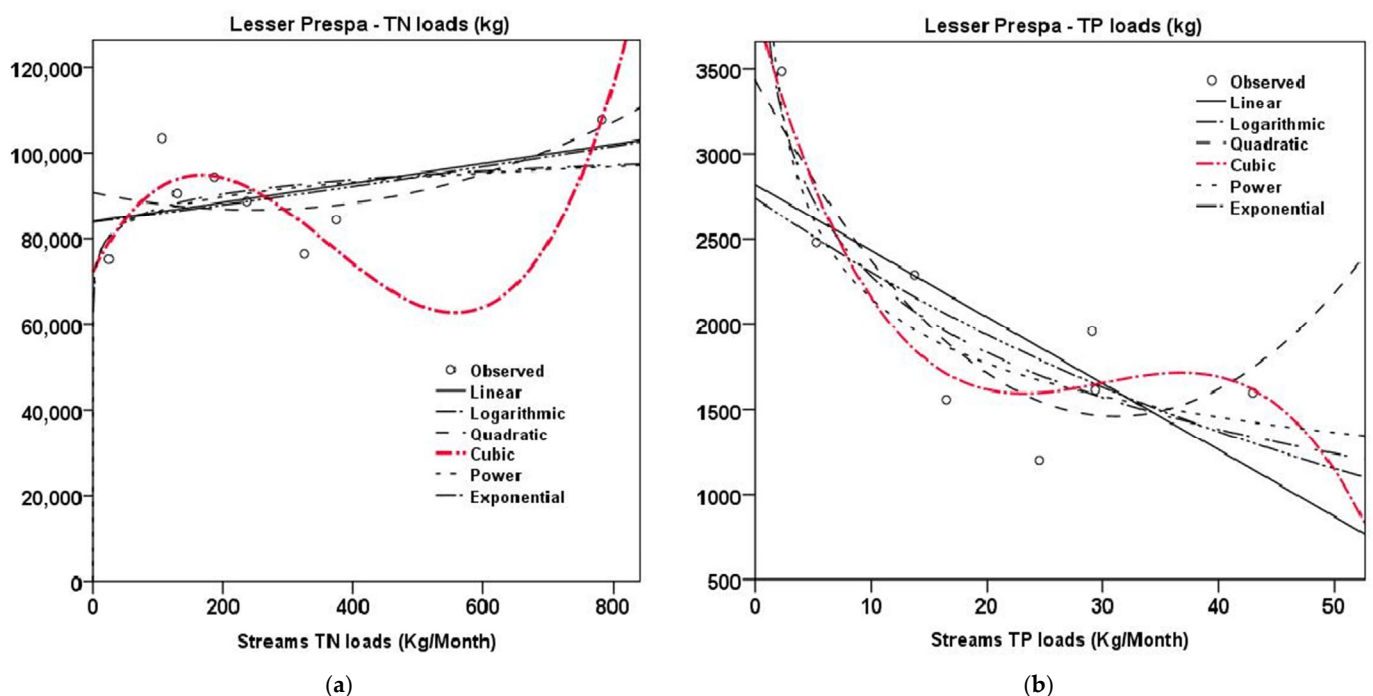
Figure 6. Temporal variation of (a) TN and (b) TP loads and scatterplot of mean (c) TN loads and (d) total phosphorus loads in Lesser Prespa Lake and streams. Stream TN peaks (December 2022, February 2023) preceded lake TN rises (January, March 2023), while declines (April–May 2023) matched lower lake levels. TP followed a similar, weaker pattern.

Correlation analyses further revealed the complexity of nutrient dynamics in the lake. TN exhibited moderate associations between stream and lake concentrations, whereas TP showed a strong inverse relationship, highlighting the importance of in-lake processes such as sedimentation, sediment resuspension and algal uptake. Specifically, the Pearson correlation analysis indicated a moderate, non-significant correlation for TN ($R = 0.61$; Figure 6c) and a strong negative correlation for TP ($R = -0.94$; Figure 6d), suggesting substantial nutrient retention and processes within the lake.

Time-lagged analysis of monthly stream nutrient loads and subsequent lake concentrations showed stronger correlations with cubic regression models (Table 3). For total nitrogen, R^2 reached 0.73, while total phosphorus was even higher at $R^2 = 0.84$ (Figure 7), indicating a notable influence of streams on Lesser Prespa Lake's water quality despite their low hydrological input. The limited dataset (8 pairs), though, restricts statistical reliability, so future studies should expand sampling locations and extend monitoring beyond one year.

Table 3. Regression analysis for total nitrogen and total phosphorus between lakes' and streams' concentrations.

Dataset	Equation	R ²	F	df1	df2	p-Value	Constant	b ₁	b ₂	b ₃
TN	Linear	0.21	1.58	1	6	0.256	84,040.29	22.50		
TN	Logarithmic	0.18	1.35	1	6	0.289	64,858.93	4837.23		
TN	Quadratic	0.31	1.13	2	5	0.393	90,858.10	−32.80	0.067	
TN	Cubic	0.73	3.60	3	4	0.124	72,248.96	302.34	−1.18	0.001
TN	Power	0.19	1.38	1	6	0.285	67,414.06	0.054		
TN	Exponential	0.19	1.42	1	6	0.278	83,861.16	0.000		
TP	Linear	0.54	7.01	1	6	0.038	2821.17	−38.99		
TP	Logarithmic	0.79	22.74	1	6	0.003	3773.80	−647.78		
TP	Quadratic	0.78	9.09	2	5	0.022	3437.89	−127.01	2.04	
TP	Cubic	0.84	6.98	3	4	0.046	3860.44	−247.65	8.71	−0.10
TP	Power	0.71	14.65	1	6	0.009	4133.78	−0.28		
TP	Exponential	0.50	6.01	1						

**Figure 7.** Scatter plots showing relationships between stream and lake nutrient loads for (a) TN and (b) TP, with a one-month time lag across different regression models. Stream inputs strongly influence Lesser Prespa Lake's nutrient concentrations, with TN and TP showing high correlations ($R^2 = 0.73$ and 0.84 , respectively) despite the streams' relatively low flow.

Regarding phosphorus concentrations in lakes, it has been observed that increased levels result in reduced Secchi depth, effectively increasing water turbidity. Specifically, according to earlier studies, phosphorus loads in lakes do not originate solely from surface runoff in the watershed and transport via streams. Ref. [83] demonstrated that a portion of phosphorus is associated with suspended particles resulting from soil erosion. Ref. [16], in their effort to quantify the contribution of external loadings and internal hydrodynamic processes to the water quality of Lake Okeechobee (Central Florida), concluded that significant emphasis should be placed on understanding the lake's internal processes. This aspect is often overlooked, as most studies focus on water and nutrient discharges from watersheds. Statistical analyses and simulation models, using data from 19 sampling points within the lake, revealed that water quality in Lake Okeechobee depends primarily on factors such as air temperature, concentrations of TP entering the lake from nearby rivers, and the amount

of water exiting the lake. However, the significance of these factors varied depending on the sampling station. Regarding internal hydrodynamic processes, scientists found that wind speed (horizontal currents) had the greatest impact on algal biomass, surpassing the influence of riverine discharges.

Additionally, according to [84], in river–lake systems, rivers act as nutrient carriers, while lakes serve as temporary reservoirs for nutrient accumulation. The deposition processes of these nutrients in bottom sediments depend on the physicochemical conditions of each lake. Simultaneously, nutrient salts are consumed by primary producers during their growth period, reducing their concentrations in lakes [85–87].

Finally, ref. [17] studied the variability in the concentration and forms of nitrogen and phosphorus in the waters of the Głuszynka River (24.4 km long) and eight lakes in the Kórnik–Zaniemyśl Gutter (central-western Poland). The researchers indicated that, based on nitrogen biogeochemistry, its concentration is expected to increase along the river. Conversely, phosphorus, which precipitates easily, decreases in concentration until it reaches the lakes, where it is characterized by longer residence times in the water (over one year).

All of the aforementioned studies emphasize the importance and the necessity of considering both the internal hydrodynamic processes (and the physicochemical conditions at multiple stations) and the external loadings in Lesser Prespa Lake to gain a comprehensive understanding of its water quality.

3.3. Estimation of Water Balance Components for Prespa Lakes

3.3.1. Lesser Prespa Lake

Figure 8 presents the temporal variation in the estimated water balance components, namely, direct precipitation, evaporation and surface inflows to the Lesser Prespa, along with the corresponding changes in water level and lake storage over the study period (November 2022 to October 2023).

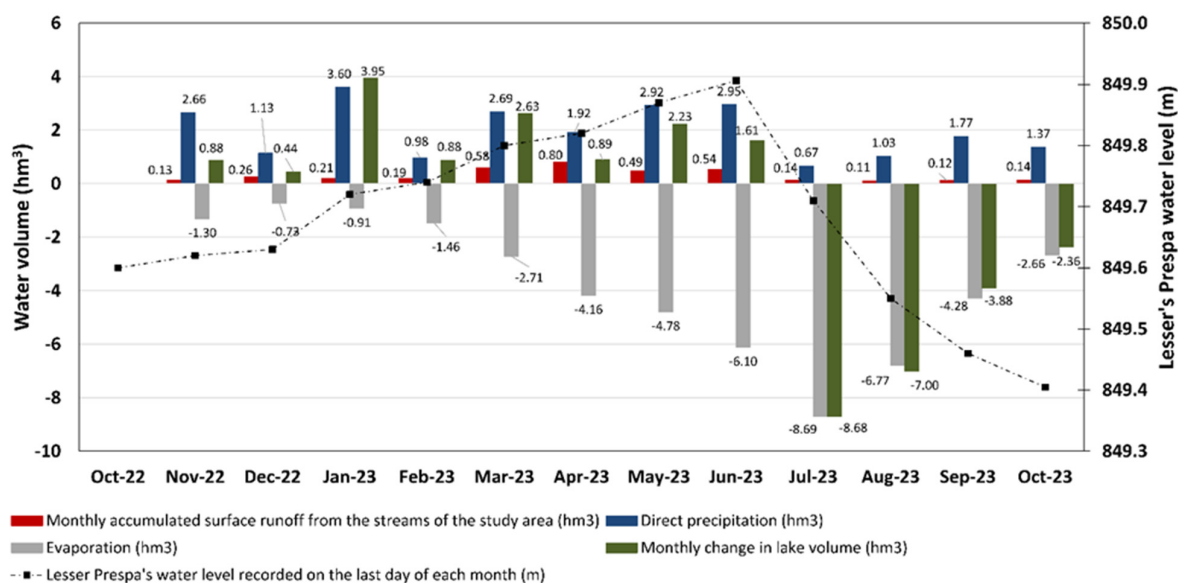


Figure 8. Temporal variation in the lake's surface elevation (m) and in the water balance components (hm³) examined for the Lesser Prespa throughout the 1-year study period (2022–2023).

The lake exhibited a negative water balance on a monthly basis for the period between July and October 2023. Over this four-month period, the cumulative reduction in lake volume exceeded 20 hm³, indicating a substantial water deficit during the dry season. In fact, the latter reduction in lake storage appeared to be well correlated with the water

volume loss due to evaporation (Figure 8), indicating that the latter is the dominant process affecting water storage over this period of years. Over the rest of the study period, positive changes in the lake storage were observed, varying between $+0.44 \text{ hm}^3$ (December) and $+3.95 \text{ hm}^3$ (January). Seasonal snowmelt led to comparatively increased inflows during spring; in particular, the maximum surface runoff observed in April 2023 was estimated at about 0.8 hm^3 , a contribution which translates into a potential 2 cm rise in the lake level. The analysis revealed that Mikrolimni and Kallithea streams provided the largest inflows among the examined catchments, collectively accounting for 80% of the annual surface contribution. In contrast, the Plateos–Milionas stream contributed the least to the annual surface inflow, representing barely 5% of it.

On an annual scale, Lesser Prespa Lake exhibited a negative water balance, with a total volume loss of approximately 8.4 million cubic meters (hm^3) and a water surface elevation decline of $\sim 20 \text{ cm}$ over the study period (November 2022–October 2023). On a hydrologic-year level (October 2022–September 2023), the lake's level decreased by 16 cm, with a corresponding volume loss of 6.9 hm^3 . Over the study period, surface outflow from the study catchment was estimated at about 3.7 hm^3 and rainfall volume at $\sim 23.7 \text{ hm}^3$, whereas losses due to evaporation reached 44.6 hm^3 . The surface outflow estimated for the Greek portion of the lake's catchment diverges markedly from that reported in a previous hydrological assessment of the area [88]. In the latter study, titled "Feasibility Study, Project Preparation & Development of the Transboundary Prespa Park Project", the mean annual surface outflow to Lesser Prespa, derived through rainfall–runoff modeling, was estimated at 78.8 hm^3 , considering the entire contributing watershed ($\sim 218.9 \text{ km}^2$) and rainfall data for the period of 1951–2004. Since the present study accounts for only 17% of the total watershed (i.e., the portion within Greek territory), the runoff estimates produced herein differ by approximately 60% from those reported in the KfW analysis [88]. This yet pronounced discrepancy likely reflects uncertainties inherent in the earlier modeling effort, particularly due to the absence of calibration data and the necessary transfer of hydrological parameters from the Ag. Germanos basin.

To explore interactions and identify potential relationships between the recorded changes in lake volume and the examined water balance components, a Pearson correlation analysis was conducted. Monthly values of the variables were analyzed in pairs across three different periods: (a) the entire study period ($N = 12$), (b) the wet season (October–March; $N = 6$), and (c) the dry season (April–September; $N = 6$). The analysis was limited to linear correlations, as strong linear relationships were identified, making the exploration of nonlinear dependencies unnecessary. The statistical significance of the derived relationships was evaluated considering a significance level of $p = 0.05$. In addition to precipitation, surface runoff, and evaporation, the analysis also incorporated excess rainfall—expressed as the difference between rainfall and evaporation—as it is expected to better capture the atmospheric contribution to lake storage fluctuations. The results are shown in Figure 9 in the form of correlation charts.

The analysis revealed that throughout the study period, and particularly during the wet season, changes in the lake's storage were primarily driven by excess rainfall, defined as the surface precipitation minus evaporation ($r = 0.84$, $p < 0.05$, for the entire period and $r = 0.77$ for the wet season). In contrast, the correlation between lake volume fluctuations and inflows from the study area was proved to be notably lower when considering the entire study period ($r = 0.53$) and wet season ($r = 0.45$). This weaker relationship is likely attributed to the relatively low inflow levels of the examined streams combined with a potential lag in the lake's hydrological response, which both hamper the identification of remarkable correlations. Conversely, the uniform contribution of direct precipitation and

evaporation on the entire lake surface appeared to exert an immediate influence on water level fluctuations.

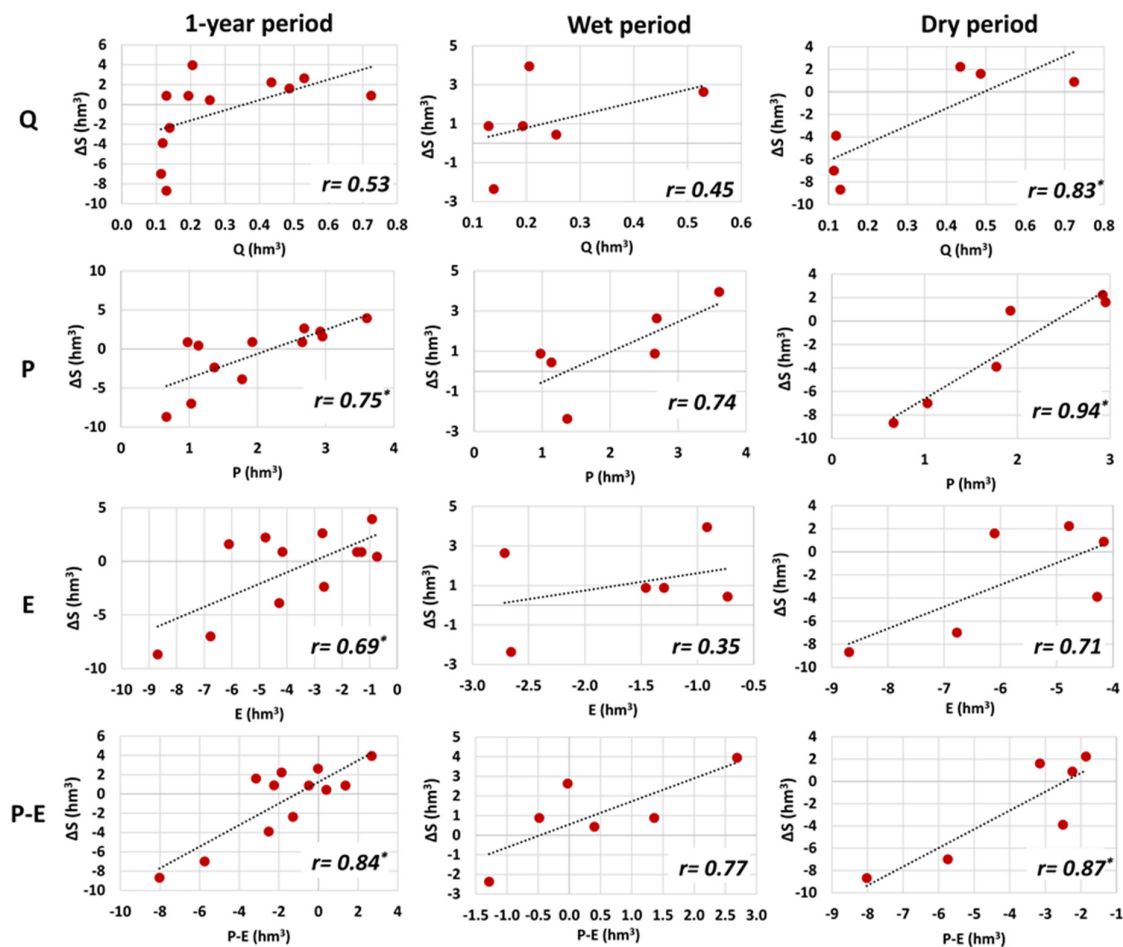


Figure 9. Correlation charts between monthly change in the Lesser Prespa's lake storage (ΔS) and each of the water balance components examined, i.e., surface runoff from the catchments of the study area (Q), direct precipitation (P), evaporation from the lake surface (E) and precipitation–evaporation ($P-E$) difference, for the entire 1-year period (November 2022–October 2023) and separately for the wet (October–March) and dry (April–September) periods. Statistically significant correlations at $p = 0.05$ are indicated by an asterisk.

It is interesting to note, though, that most of the studied components exhibited strong and statistically significant ($p < 0.05$) correlations with changes in lake volume during the dry season. This indicates that the decline in water storage over this period is the aggregate effect of both the exceptionally low inflows (precipitation and runoff) and the high evaporation rates. Additionally, although not explicitly quantified here, water abstraction for irrigation during the summer season is expected to have exerted further pressure, exacerbating the decline in water levels.

3.3.2. Great Prespa Lake

The presence of the Ag. Germanos automatic monitoring station, prior to the initiation of the current survey, enabled the investigation of the river's influence on the water storage of Great Prespa over a longer time period. Consequently, a three-year period from October 2020 to September 2023 was analyzed, incorporating the same water balance components as in the Lesser Prespa analysis, namely, direct rainfall, evaporation, and outflow from the Ag. Germanos River. The analysis was performed on a monthly basis, while results were

also aggregated at the hydrologic-year scale (October to September). Figure 10 displays the variation in the examined water balance components in parallel with lake storage change and water elevation over the study period.

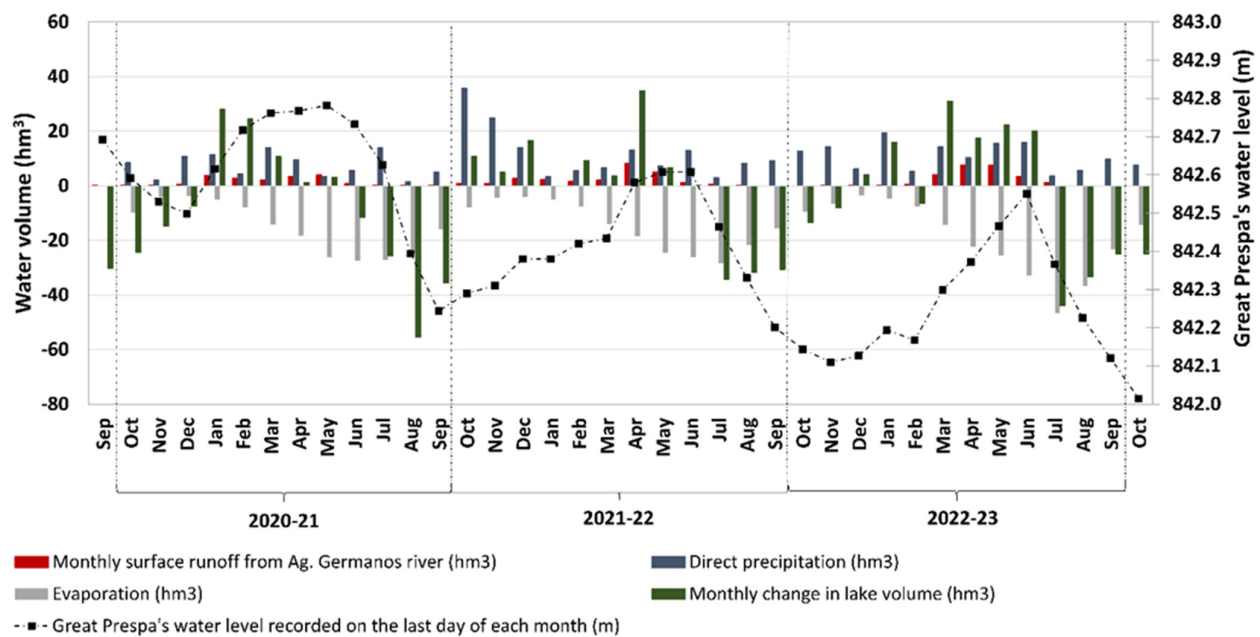


Figure 10. Temporal variation in the lake's surface elevation (m) and in the water balance components examined (hm^3) for the Great Prespa throughout the 3-year period (2020–2023) [35].

The greatest outflows from Ag. Germanos are typically observed between April and May every year, influenced by the snowmelt, while the highest water levels of the Great Prespa Lake generally occur with an approx. one-month lag, between May and June. The overall maximum monthly outflow to the lake was recorded in April 2022 and exceeded 8 hm^3 , a contribution which may be translated to a theoretical 3.5 cm increase in the lake's water stage. In fact, during the spring season, Ag. Germanos was found to significantly contribute to the lake storage, discharging water quantities comparable to those contributed by the surface rainfall.

A sharp decline in the lake's water volume is apparent during summer, with the greatest water loss recorded in August 2021 and corresponding to approx. 55 hm^3 . This substantial water loss seems to be primarily attributed to the negligible inflows from Ag. Germanos catchment during this period, compounded by both natural factors—namely, the comparatively low precipitation and elevated evaporation rates—and anthropogenic influences, particularly substantial water abstractions for irrigation.

Throughout the study period, annual losses exceeded gains, leading to a systematic decline in water volume of the Great Prespa. In fact, a comparative examination of both lakes' elevation over the last few years (2020–2023) revealed a more than 10-times-higher decline rate of the Great Prespa water stage compared to the Lesser Prespa, with the level of the latter remaining more or less stable ($\sim 5 \text{ cm}$ decline over the 3-year period). The subsequent increase in the water elevation difference between the neighboring lakes is of critical importance, as it translates into a systematic rise in the hydraulic gradient between their stages, rendering Lesser Prespa potentially vulnerable, due to the subsequent increase in subsurface outflow to the Great Prespa through the alluvial isthmus.

Over the three-year study period, annual volume losses from Great Prespa ranged from 10.1 hm^3 to 107.2 hm^3 , while evaporation losses varied between 177.5 hm^3 and 233.8 hm^3 and direct precipitation between 89.9 hm^3 and 144.3 hm^3 . Annual surface runoff

from the Ag. Germanos catchment was estimated to range from 18.7 hm³ to 25.7 hm³. A comparable hydrological assessment by [89] reported mean annual rainfall contributions of 159.4 hm³, mean annual evaporation of 195.3 hm³, and mean annual volume losses of 32.8 hm³ over the 1990–2008 period. In the same study, surface runoff from the entire Great Prespa catchment was estimated at 266.1 hm³. Considering that the Ag. Germanos catchment represents approximately 8% of the total basin area (65.9 km² out of 840 km²), the runoff estimates produced herein can be regarded as broadly consistent with those reported by [89], falling within the same order of magnitude.

The Pearson correlation analysis undertaken for the case of Great Prespa (Figure 11) demonstrated that the Ag. Germanos surface inflow constitutes the primary contributor among the examined components, exhibiting a strong and statistically significant positive correlation with the lake's storage variations, during both the wet ($r = 0.79$, $p < 0.05$) and dry ($r = 0.88$, $p < 0.05$) periods.

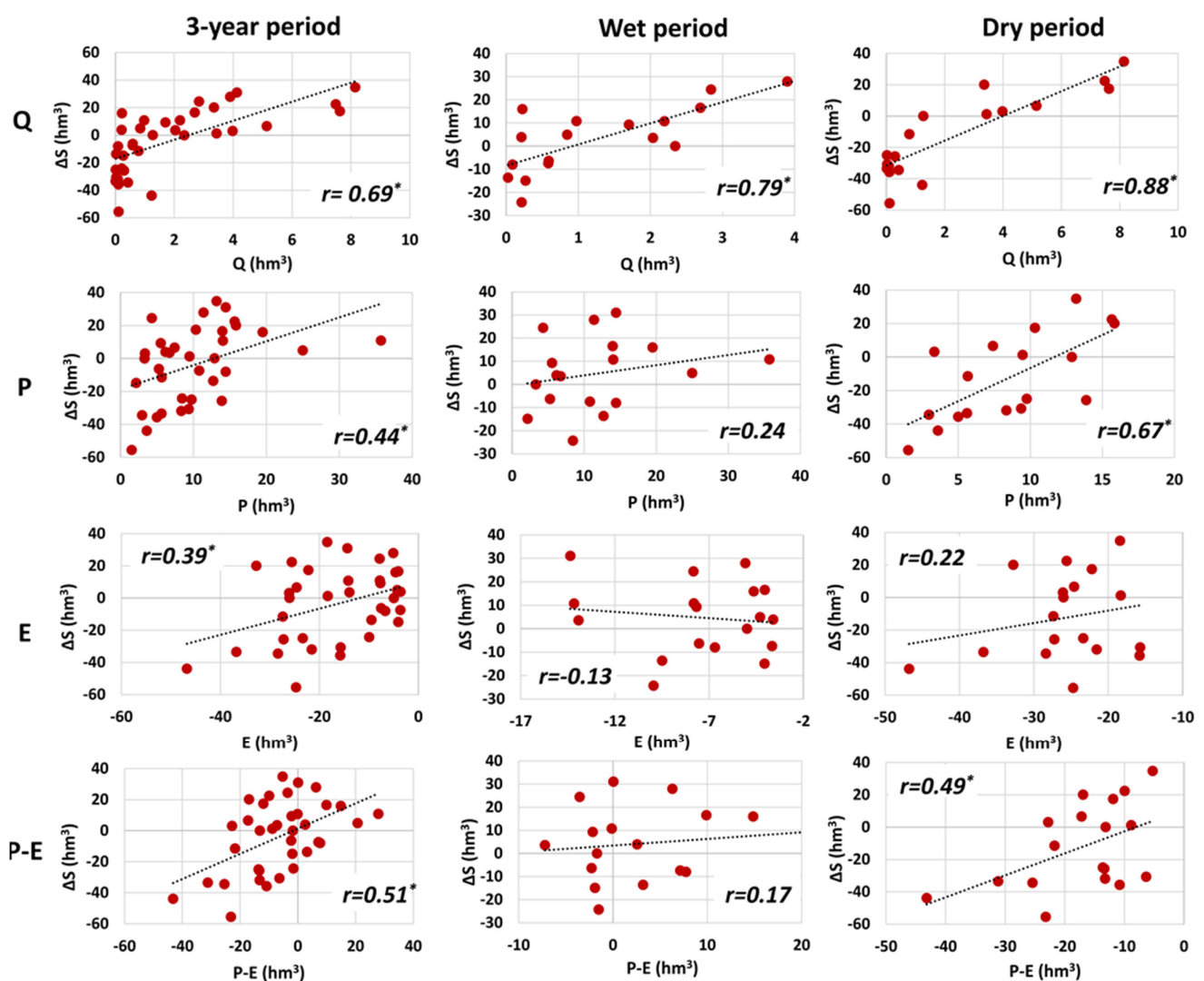


Figure 11. Correlation charts between monthly change in the Great Prespa's lake storage (ΔS) and the water balance components examined, i.e., surface runoff from Ag. Germanos river (Q), direct precipitation (P), evaporation from the lake surface (E) and precipitation–evaporation ($P-E$) difference, for the entire 3-year period ($N = 36$) and separately for the wet (October–March; $N = 18$) and dry (April–September; $N = 18$) periods. Statistically significant correlations at $p = 0.05$ are indicated by an asterisk.

Interestingly, direct rainfall exhibited much lower correlation with the changes in the lake volume, with a moderate positive relationship ($r = 0.67$, $p < 0.05$) identified only for the dry period, whereas for the wet season and the entire study period, the resulting correlations were rather weak. This finding, though, is consistent with previous results [90], where a weak correlation was also reported between rainfall and water storage change on a monthly basis, primarily attributed to the complex interactions with the groundwater aquifer system.

3.4. Water Stable Isotopes

The water isotope data for river waters revealed mean values of -8.5‰ for $\delta^{18}\text{O}$ and -57.1‰ for $\delta^2\text{H}$ in autumn and -9.4‰ for $\delta^{18}\text{O}$ and -63.3‰ for $\delta^2\text{H}$ in winter (Figure 12). In lake waters, the mean values were -0.5‰ for $\delta^{18}\text{O}$ and -13.6‰ for $\delta^2\text{H}$ in autumn and -1.0‰ for $\delta^{18}\text{O}$ and -17.4‰ for $\delta^2\text{H}$ in winter [35]. The d -excess values in rivers were generally higher ($\sim +12.0\text{‰}$) than those observed in the lakes ($< +10.0\text{‰}$). Based on the relationship between $\delta^{18}\text{O}$ and altitude [91], river recharge is estimated to originate from an average altitude of 900–950 m.

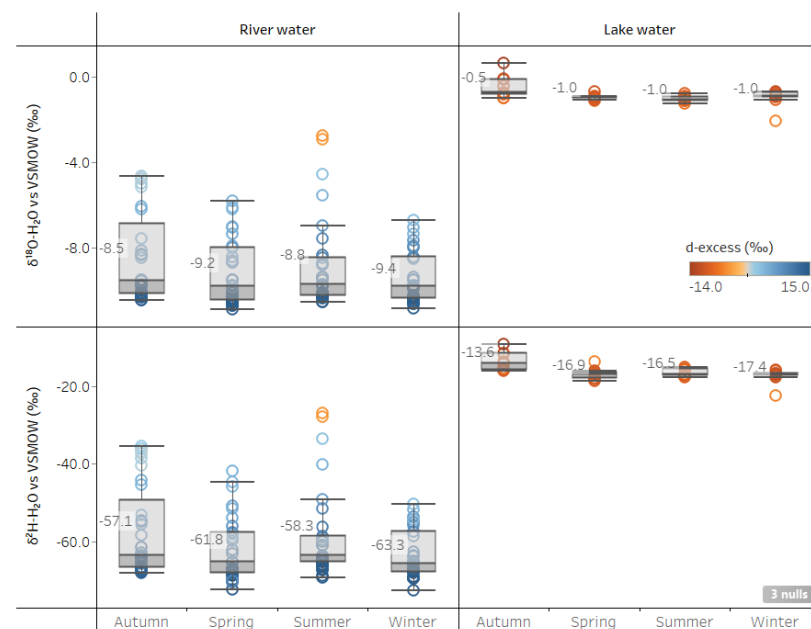


Figure 12. Seasonal patterns in water isotope composition differ among water types. Water $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values for river water and lake water, grouped by season, based on $n = 152$ samples collected between 2022 and 2023 (source: [35]).

Great Prespa and Lesser Prespa Lakes showed no significant differences in their mean isotopic composition (p -value > 0.05) with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of -0.9‰ and -16.0‰ for Great Prespa Lake and -1.4‰ and -19.4‰ for Lesser Prespa Lake, respectively. This indicates that both lakes are likely recharged from similar source(s) and experience similar physical processes. The notably low d -excess values in both lakes suggest the influence of fractionation due to evaporation.

Agios Germanos River, a perennial river discharging into the Great Prespa Lake, showed the lowest mean $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values (-10.2‰ and -66.7‰ , respectively) compared to the other rivers of the basin [35], likely reflecting differences in recharge altitude and/or evaporation effects. The river's headwaters originate at ~ 2000 m, where spring discharge occurs. In contrast, rivers, such as Milonas and Karies, which flow into the Lesser Prespa Lake, have intermittent flow, enhancing isotopic fractionation due to evaporation—particularly during the dry season, when the river flow is small. Along the course of

Ag. Germanos River, the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values were almost constant (Ag. Germanos 2–6) but increased downstream near its outflow into the Great Prespa Lake (Ag. Germanos 1; Figure 13), possibly due to local discharge or mixing with the lake water. Mikrolimni River, which flows into the Lesser Prespa Lake, showed statistically insignificant differences in the water isotope values (-7.3‰ and -52.0‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively) between upstream (Mikrolimni_3) and downstream (Mikrolimni_1) sampling stations. These differences between the two rivers are primarily attributed to evaporation effects, as indicated by the d -excess values, altering the initial isotopic composition.

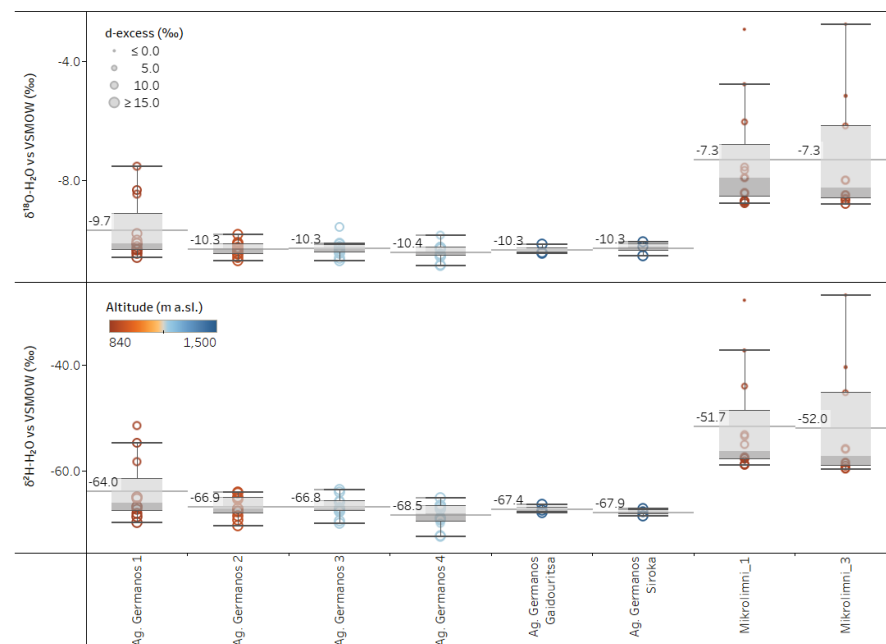


Figure 13. Longitudinal patterns in water isotope composition reveal spatial variability along the two rivers. Water $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values along the Ag. Germanos and Mikrolimni rivers from upstream (Ag. Germanos 6 and Mikrolimni_3) to downstream (Ag. Germanos 1 and Mikrolimni_1), based on $n = 88$ samples collected during 2022–2023 (source: [35]).

The $\delta^{18}\text{O}$ – $\delta^2\text{H}$ relationship showed that river water samples cluster close to the Local Meteoric Water Line (LMWL), indicating a dominant meteoric origin with limited evaporative enrichment apart from those river water samples that deviated from the LMWL (e.g., Milonas, Figure S1 in the Supplementary File). In contrast, lake water samples plotted along the Local Evaporation Line (LEL) and were clearly shifted towards heavier isotopic values, reflecting the influence of evaporation [35]. The alignment of the lake waters with a mixing trend extending from the river samples indicated partial mixing between river inflows and lake water. This suggests that river discharge contributes to lake water composition, which is progressively enriched by evaporation.

The Bayesian mixing model results (Figure 14) showed that in the wet period, the relative contribution of river waters to the recharge of the Great Prespa Lake was highest ($\sim 60\%$), followed by precipitation ($\sim 40\%$). In the dry period, the relative contribution of precipitation was higher ($\sim 60\%$) than the river water ($\sim 40\%$). The relative contribution of the Lesser Prespa Lake to the recharge of the Great Prespa Lake was small ($<10\%$) in both periods. Overall, these findings corroborate the inference previously drawn from the correlation analysis for the Great Prespa Lake (Figure 11), particularly highlighting the comparatively greater influence of Agios Germanos inflow on the lake's water storage during the wet season. However, in the dry season, the mixing model results (Figure 14) showed a slightly opposite pattern compared to the earlier correlation results (Figure 11),

suggesting a higher contribution from precipitation relative to river inflows. This discrepancy may be partly attributed to methodological discrepancies related to data origin, such as the rainfall data. It should also be noted that the isotopic data used in the mixing model include records not only from the monitoring period but also from earlier studies, which may also contribute to this discrepancy.

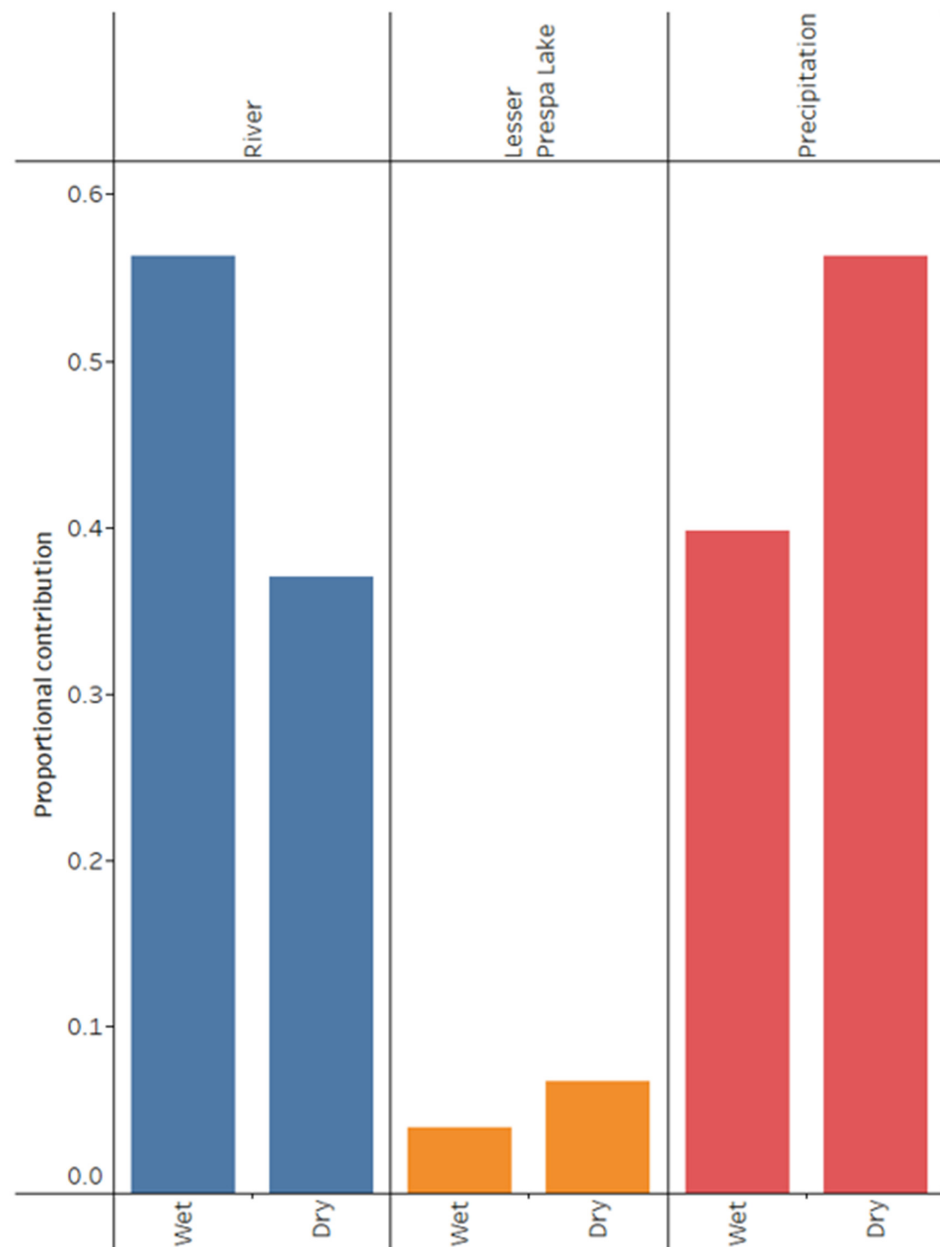


Figure 14. Posterior mean estimates of water source contributions to Great Prespa Lake show strong seasonal variability. Results of the isotope mixing model showing the proportional contributions of river inflow, Lesser Prespa Lake and precipitation during wet and dry periods (source: [35]).

In the water balance analysis, rainfall data were obtained from the Koula meteorological station (Figure 1), situated at the southern boundary of the lake, which may have introduced some uncertainty in capturing the rainfall spatial variability across the lake's extensive surface area. On the other hand, the rainfall isotopic data were obtained from the PisoAI model [92], given the absence of rainwater samples. Nonetheless, despite this minor inconsistency, both approaches seem to highlight the increasing impor-

tance of the rainfall for the Great Prespa's recharge over the dry season, which is evident through its comparatively higher proportional contribution relative to river inflow (Figure 14), as well as its stronger correlation with the lake storage when compared to the wet period (Figure 11).

4. Conclusions and Recommendations

The present study highlights the ecological and hydrological significance of the Prespa Lakes system, a transboundary ecosystem shared by Greece, Albania, and the Republic of North Macedonia. Focused on the Greek part of the Prespa basin, the results emphasize the limited availability of comprehensive monitoring data, underlining the need for further research and integrated, basin-wide management approaches.

More specifically, the analysis of nutrient concentrations, alongside biological and water quality and quantity parameters, indicates that seasonal and spatial variations are strongly influenced by both natural hydrological dynamics and anthropogenic pressures within the basin. These findings reinforce the importance and the necessity of adopting holistic, cross-border management strategies that consider the interconnectedness of Prespa Lakes and their surrounding catchments while leading to fundamental key observations in terms of water quality, quantity and ecological aspects:

- Streams such as Agios Germanos maintain high ecological quality, serving as a critical reference for conservation efforts.
- Moderate ecological status at certain stations highlights local stress hotspots linked to nearby agricultural, livestock, irrigation, and wastewater pressures.
- Repeated high nutrient levels underscore the need to investigate nearby pollution sources and optimize contributing practices.
- Lesser Prespa Lake is more vulnerable to nutrient enrichment.
- Understanding internal hydrodynamics (e.g., pollutant retention and transformation in the littoral zone and sediments) and the lake's physicochemical conditions is essential. In situ sediment measurements and/or modeling approaches are vital in order to gain an in-depth understanding of the long-term responses of internal environmental dynamics in a freshwater lake to fluctuations in external nutrient inputs.
- Riverine nutrient concentrations can strongly influence lake water quality even though this was not substantially obvious in the current study due to climatic, hydrologic and limnologic reasons that were analytically explained above. Distinct drivers were found to control storage changes in the two lakes, with Lesser Prespa being primarily influenced by excess rainfall ($R = 0.77\text{--}0.87$), and Great Prespa by inflows from Ag. Germanos River ($R = 0.69\text{--}0.88$).

Our analysis is inevitably subject to a certain number of limitations, which should be acknowledged. Among them, the most critical ones are presented below:

- **Limited Capacity of Capturing Interannual Variability:** The present study represents an interdisciplinary effort, involving an extensive and resource-intensive sampling campaign, for the collection of a wide range of physicochemical, biological, and hydrological parameters, in order to provide a holistic evaluation from both ecological and quantitative perspectives. As such, the analysis primarily focused on a particular hydrologic year for which all required data were available. The derived findings are, thus, mostly indicative of the hydrological year under investigation and are primarily able to capture seasonal variability rather than interannual dynamics. It should be noted, though, that for the case of Great Prespa, a relatively longer period (three hydrological years) has been examined, owing to the availability of discharge data from an automatic hydrological station located on the Ag. Germanos River, which enabled a more extended interpretation of the lake's water balance.

- **Non-Incorporation of Groundwater Component in Water Balance Analysis:** In the present study, only selected water balance components were explicitly investigated, namely surface inflow from the study area, direct precipitation, evaporation, and storage changes in the Prespa Lakes. Consequently, groundwater exchange was not quantified, although it is known to play a substantial role in lake storage fluctuations, particularly in Great Prespa Lake. This methodological choice was dictated by data availability and by the fact that groundwater exchanges are typically estimated indirectly, requiring comprehensive quantification of all other water balance components. Such a comprehensive investigation was not feasible herein, as the study focuses exclusively on the Greek part of the Prespa Lakes, leaving a significant portion of surface runoff from neighboring countries unmonitored and unknown, thereby precluding a reliable estimation of the groundwater component.
- **Uncertainty in Water Balance Estimation:** The water balance results derived in this study are inevitably subject to uncertainties arising from both data limitations and methodological assumptions. Within this context, simplified water balance approaches, although widely used in water resources management, cannot fully capture the complexity of real-world hydrological dynamics, while key processes such as groundwater–surface water interactions and internal lake processes are not explicitly represented. With regard especially to our estimates, additional uncertainty may be associated with the use of meteorological data from a single station to estimate precipitation and evaporation over the extensive lake surfaces, thus assuming spatially uniform meteorological conditions and potentially underrepresenting spatial variability, particularly in the case of Great Prespa. Uncertainties related to inflow estimation may also have affected the results, due to limitations in both in situ discharge measurements and rating-curve-based estimates, although the latter exhibited strong agreement with observed data ($R^2 = 0.83\text{--}0.93$).
- **Lack of Quantification of Internal Nutrient Cycling:** Internal nutrient cycling can be assessed or quantified using either empirical measurements or modeling approaches [93]. Empirical measurements are commonly obtained through the collection of undisturbed sediment cores with intact overlying water, which are subsequently analyzed under controlled laboratory conditions designed to replicate in situ environmental settings. As a shortcoming of the present study, neither sediment samples were collected, nor alterations in the internal environment under various degrees of external nutrient load reduction have been compared, preventing the quantification of internal nutrient cycling.

Overall, this work provides a scientific basis for future conservation efforts, supports the sustainable use of water resources, and contributes to maintaining biodiversity and ecosystem services within the Prespa basin. It can serve as a framework for policymakers and researchers to guide collaborative initiatives in transboundary lake management.

Sustainable management of the Prespa basin requires a holistic approach integrating hydromorphology, water quantity, and quality across streams and lakes. Stream restoration should enhance habitat connectivity, maintain ecological flows, and replace summer irrigation withdrawals with efficient drip systems to protect sensitive species such as *Salmo peristericus*. For Lakes Lesser and Great Prespa, systematic monitoring of water levels, sediment composition, water balance, and key physicochemical parameters at representative stations and depths is essential. Minimum water levels in Lake Lesser Prespa should be established to preserve ecosystem functionality. Complementary measures include treatment of livestock wastewater, installation of manure collection systems, and mitigation of nutrient-rich runoff using eco-compatible methods. This integrated framework ensures ecological continuity, resilience, and long-term sustainability, providing a model

for catchment-scale water and ecosystem management in complex transboundary systems. Without such interventions, the cumulative pressures are likely to sustain high nutrient loads, where already present, with long-term consequences for the ecological status of inflowing streams and by extension the integrity and trophic status of the Prespa Lakes.

Finally, the integration of remote sensing and modeling tools, combined with coordinated transboundary initiatives, is essential for establishing standardized protocols and implementing effective management strategies that safeguard the ecological integrity and ecosystem services of the Prespa Lakes basin.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18040518/s1>, Table S1: Average temperature and monthly rainfall at Koula meteorological station (40° 48' 35.9" N, 21° 4' 14.4" E; Figure 1) over the study period; Table S2: Nutrient-and DO-based physicochemical quality classes [57,58]; Table S3: Water quality classes for Deep (>3 m) and Shallow (<3 m) lakes with total alkalinity >0.2 meq/L [64]; Table S4: Lake trophic status classification [65]; Table S5: Carlson's trophic state index values and classification of lakes [67]; Table S6: Dissolved oxygen and nutrient concentrations at all stations of rivers/streams discharging into the Prespa Lakes during November–December 2022 and January–October 2023; Table S7: Ecological data, HESY2 (EQR) values of the benthic macroinvertebrate quality index, and water quality at the sampling sites; Table S8: Ecological Quality Ratio (EQR) values of the benthic diatom quality index and water quality at the sampling sites during spring and summer; Table S9: Nutrient concentrations during the WET season at all stations of the studied rivers/streams discharging into the Prespa Lakes and their physicochemical quality and ecological status; Table S10: Nutrient concentrations during the DRY season at all stations of the studied rivers/streams discharging into the Prespa Lakes and their physicochemical quality; Figure S1: Lake waters exhibit isotopic enrichment and deviation from the Local Meteoric Water Line (LMWL: $\delta^2\text{H-H}_2\text{O} = 7.3 \delta^{18}\text{O-H}_2\text{O} + 6.1$, [27]) due to evaporation. Dual-isotope plot of $\delta^{18}\text{O-H}_2\text{O}$ versus $\delta^2\text{H-H}_2\text{O}$ for lake (orange, $n = 36$) and river (blue, $n = 116$) samples collected during 2022–2023, with the LMWL and the Local Evaporation Line (LEL: $\delta^2\text{H-H}_2\text{O} = 5.6\delta^{18}\text{O-H}_2\text{O} + 12.2$, [27]) shown for reference (source: [35]).

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References

- Hirji, R.F.; Duda, A. Integrated management of lakes, reservoirs, and their basins is critical for a climate-resilient planet: An urgent wake-up call from collective amnesia. *Water Policy* **2025**, *27*, 66–87. [\[CrossRef\]](#)
- Meadows, G.A.; Meadows, L.A.; Wood, W.L.; Hubertz, J.M.; Perlin, M. The relationship between Great Lakes water levels, wave energies, and shoreline damage. *Bull. Am. Meteorol. Soc.* **1997**, *78*, 675–682. [\[CrossRef\]](#)
- Khan, S.J.; Deere, D.; Leusch, F.D.; Humpage, A.; Jenkins, M.; Cunliffe, D. Extreme weather events: Should drinking water quality management systems adapt to changing risk profiles? *Water Res.* **2015**, *85*, 124–136. [\[CrossRef\]](#) [\[PubMed\]](#)
- Casanova, M.T.; Brock, M.A. How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecol.* **2000**, *147*, 237–250. [\[CrossRef\]](#)
- Munar, A.M.; Cavalcanti, J.R.; da Motta-Marques, D.; Fragoso, C.R. Coupling large-scale hydrological and hydrodynamic modeling: Toward a better comprehension of watershed-shallow lake processes. *J. Hydrol.* **2018**, *564*, 424–441. [\[CrossRef\]](#)
- Kowalczywska-Madura, K.; Dondajewska-Pielka, R.; Gołdyn, R. The assessment of external and internal nutrient loading as a basis for lake management. *Water* **2022**, *14*, 2844. [\[CrossRef\]](#)
- Xu, H.; McCarthy, M.J.; Paerl, H.W.; Brookes, J.D.; Zhu, G.; Hall, N.S.; Qin, B.; Zhang, Y.; Zhu, M.; Justyna, J.; et al. Contributions of external nutrient loading and internal cycling to cyanobacterial bloom dynamics in Lake Taihu, China: Implications for nutrient management. *Limnol. Oceanogr.* **2021**, *66*, 1492–1509. [\[CrossRef\]](#)
- Yang, C.; Yang, P.; Geng, J.; Yin, H.; Chen, K. Sediment internal nutrient loading in the most polluted area of a shallow eutrophic lake (Lake Chaohu, China) and its contribution to lake eutrophication. *Environ. Pollut.* **2020**, *262*, 114292. [\[CrossRef\]](#)
- Qin, B.; Zhang, Y.; Zhu, G.; Gao, G. Eutrophication control of large shallow lakes in China. *Sci. Total Environ.* **2023**, *881*, 163494. [\[CrossRef\]](#)
- Qiu, J.; Yuan, S.; Tang, H.; Zhang, Q.; Wolter, C.; Nikora, V. Ecological connectivity of river-lake ecosystem: Evidence from fish population dynamics in a connecting channel. *Water Resour. Res.* **2024**, *60*, e2024WR037495. [\[CrossRef\]](#)
- World Meteorological Organization. *State of the Global Water Resources Study of 2023*; WMO-No. 1333; WMO: Geneva, Switzerland, 2023. Available online: <https://library.wmo.int/records/item/68473-state-of-global-water-resources-report-2022> (accessed on 25 October 2025).
- Röpke, C.; Pires, T.H.; Zuchi, N.; Zuanon, J.; Amadio, S. Effects of climate-driven hydrological changes in the reproduction of Amazonian floodplain fishes. *J. Appl. Ecol.* **2022**, *59*, 1134–1145. [\[CrossRef\]](#)
- He, C.; James, L.A. Watershed science: Linking hydrological science with sustainable management of river basins. *Sci. China Earth Sci.* **2021**, *64*, 677–690. [\[CrossRef\]](#)
- Inácio, M.; Barceló, D.; Zhao, W.; Pereira, P. Mapping lake ecosystem services: A systematic review. *Sci. Total Environ.* **2022**, *847*, 157561. [\[CrossRef\]](#) [\[PubMed\]](#)
- Qi, H.; Lu, J.; Chen, X.; Sauvage, S.; Sanchez-Pérez, J.M. Water age prediction and its potential impacts on water quality using a hydrodynamic model for Poyang Lake, China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 13327. [\[CrossRef\]](#)
- Shin, S.; Her, Y.; Muñoz-Carpena, R.; Yu, X. Quantifying the contribution of external loadings and internal hydrodynamic processes to the water quality of Lake Okeechobee. *Sci. Total Environ.* **2023**, *883*, 163713. [\[CrossRef\]](#)
- Janicka, E.; Kanclerz, J.; Wiatrowska, K.; Budka, A. Variability of Nitrogen and Phosphorus Content and Their Forms in Waters of a River-Lake System. *Front. Environ. Sci.* **2022**, *10*, 874754. [\[CrossRef\]](#)
- Hillebrand, B.; Biemans, W.G. The relationship between internal and external cooperation: Literature review and propositions. *J. Bus. Res.* **2003**, *56*, 735–743. [\[CrossRef\]](#)
- Hillebrand, B.; Biemans, W.G. Links between internal and external cooperation in product development: An exploratory study. *J. Prod. Innov. Manag.* **2004**, *21*, 110–122. [\[CrossRef\]](#)
- Sondergaard, M.; Jensen, J.P.; Jeppesen, E. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* **2003**, *506*, 135–145. [\[CrossRef\]](#)
- Sondergaard, M.; Jensen, J.P.; Jeppesen, E. Internal phosphorus loading in shallow Danish lakes. In *Shallow Lakes' 98: Trophic Interactions in Shallow Freshwater and Brackish Waterbodies*; Springer: Dordrecht, The Netherlands, 1999; pp. 145–152. [\[CrossRef\]](#)
- Faridmarandi, S.; Khare, Y.P.; Naja, G.M. Long-term regional nutrient contributions and in-lake water quality trends for Lake Okeechobee. *Lake Reserv. Manag.* **2020**, *37*, 77–94. [\[CrossRef\]](#)
- Mooney, J.R.; Stanley, H.E.; Rosenthal, W.C.; Esselman, P.C.; Kendall, A.D.; McIntyre, P.B. Outsized nutrient contributions from small tributaries to a Great Lake. *Biol. Sci.* **2020**, *117*, 28175–28182. [\[CrossRef\]](#) [\[PubMed\]](#)
- Catsadorakis, G.; Roumeliotou, V.; Koutseri, I.; Malakou, M. Multifaceted local action for the conservation of the transboundary Prespa lakes Ramsar sites in Balkans. *Mar. Freshw. Res.* **2022**, *73*, 1174–1183. [\[CrossRef\]](#)
- Trajkovski, D.; Apostolova, N. The Catastrophic Water Loss of Ancient Lake Prespa: A Chronicle of a Death Foretold. *Hydrology* **2024**, *11*, 199. [\[CrossRef\]](#)
- Armitage, D.; Otter, H.S.; van Tatenhove, J.; Dewulf, G. Science–policy processes for transboundary water governance. *Environ. Sci. Policy* **2015**, *51*, 26–35. [\[CrossRef\]](#)

27. Lima-Quispe, N.; Ruelland, D.; Rabatel, A.; Lavado-Casimiro, W.; Condom, T. Modeling Lake Titicaca's water balance: The dominant roles of precipitation and evaporation. *Hydrol. Earth Syst. Sci.* **2025**, *29*, 655–682. [\[CrossRef\]](#)
28. Menció, A.; Folch, A.; Mas-Pla, J. Analyzing hydrological sustainability through water balance. *Environ. Manag.* **2010**, *45*, 1175–1190. [\[CrossRef\]](#)
29. Gichamo, T.; Abate, B.; Esimo, F.; Gokcekus, H.; Gelete, G. Analysis of water balance and hydrodynamics of Lake Beseka, Ethiopia. *J. Water Clim. Change* **2022**, *13*, 2034–2047. [\[CrossRef\]](#)
30. Matiatos, I.; Papadopoulos, A.; Panagopoulos, Y.; Dimitriou, E. Insights into the influence of morphology on the hydrological processes of river catchments using stable isotopes. *Hydrol. Sci. J.* **2023**, *68*, 1487–1498. [\[CrossRef\]](#)
31. Drougas, C.; Kelepertzis, E.; Kyritidou, Z.; Sigala, E.; Matiatos, I.; Dotsika, E.; Vasileiou, E.; Louloudis, G.; Mertiri, E.; Boeckx, P.; et al. Controls on the geochemical composition of surface water in Alfeios River basin in the transition era of lignite mine closure at Megalopolis, Greece. *Sci. Total Environ.* **2025**, *970*, 179006. [\[CrossRef\]](#)
32. Vystavna, Y.; Harjung, A.; Monteiro, L.R.; Matiatos, I.; Wassenaar, L.I. Stable isotopes in global lakes integrate catchment and climatic controls on evaporation. *Nat. Commun.* **2021**, *12*, 7224. [\[CrossRef\]](#)
33. Johnstone, C.; Bedaso, Z.K.; Ekberg, M. Characterizing surface water and groundwater interaction for sustainable water resources management in southwestern Ohio. *Sustain. Water Resour. Manag.* **2022**, *8*, 10. [\[CrossRef\]](#)
34. Salvadori, M.; Pennisi, M.; Masciale, R.; Fidelibus, M.D.; Frollini, E.; Ghergo, S.; Parrone, D.; Preziosi, E.; Passarella, G. Isotopic study for evaluating complex groundwater circulation patterns, hydrogeological zoning, and water-rock interaction in a Mediterranean coastal karst environment. *Sci. Total Environ.* **2024**, *955*, 176850. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Matiatos, I.; Papadopoulos, A.; Harjung, A.; Vystavna, Y.; Lazogiannis, K.; Rossi, P.M.; Heiderscheidt, E.; Mentzafou, A.; Zotou, I.; Dimitriou, E. Water dynamics and evaporation losses to inflows into transboundary Mediterranean lakes—The case of Prespa Lakes. *Hydrol. Sci. J.* **2025**, *70*, 611–627. [\[CrossRef\]](#)
36. Birk, S.; Bonne, W.; Borja, A.; Brucet, S.; Courrat, A.; Poikane, S.; van de Bund, W.; Zampoukas, N.; Hering, D. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the WaterFramework Directive. *Ecol. Indic.* **2012**, *18*, 31–41. [\[CrossRef\]](#)
37. Karaouzas, I.; Smeti, E.; Kalogianni, E.; Skoulikidis, N.T. Ecological status monitoring and assessment in Greek rivers: Do macroinvertebrate and diatom indices indicate same responses to anthropogenic pressures? *Ecol. Indic.* **2019**, *101*, 126–132. [\[CrossRef\]](#)
38. Morin, S.; Gómez, N.; Tornés, E.; Licursi, M.; Rosebery, J. Benthic diatom monitoring and assessment of freshwater environments: Standard methods and future challenges. In *Aquatic Biofilms: Ecology, Water Quality and Water Treatment*; Romaní, A.M., Guasch, H., Balaguer, M.D., Eds.; Academic Press: Caister, UK, 2016; pp. 111–124.
39. Hering, D.; Johnson, R.K.; Kramm, S.; Schmutz, S.; Szoszkiewicz, K.; Verdonchot, P.F.M. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: A comparative metric-based analysis of organism response to stress. *Freshw. Biol.* **2006**, *51*, 1757–1785. [\[CrossRef\]](#)
40. Johnson, R.K.; Hering, D. Response of taxonomic groups in streams to gradients in resource and habitat characteristics. *J. Appl. Ecol.* **2009**, *46*, 175–186. [\[CrossRef\]](#)
41. Eftimi, R.; Stevanović, Z.; Stojov, V. Hydrogeology of Mali Thate–Galičica karst massif related to the catastrophic decrease of the level of Lake Prespa. *Environ. Earth Sci.* **2021**, *80*, 708. [\[CrossRef\]](#)
42. FAO/UNESCO. *Carte Bioclimatique de la Zone Méditerranée: Notice Explicative*; O.N.M.: Paris, France, 1963.
43. Papadakis, J. Climates of the world. In *Their Classification Similarities, Differences and Geographic Distribution*; Libro Edicion Argentina: Buenos Aires, Argentina, 1970.
44. Hollis, G.E.; Stevenson, A.C. The physical basis of the Lake Mikri Prespa systems: Geology, climate, hydrology and water quality. *Hydrobiologia* **1997**, *351*, 1–19. [\[CrossRef\]](#)
45. Popovska, C.; Bonacci, O. Basic data on the hydrology of Lakes Ohrid and Prespa. *Hydrol. Process.* **2007**, *21*, 658–664. [\[CrossRef\]](#)
46. Kiri, E. Hydrodynamic and Hydrochemical Investigation of the Transboundary Aquifer System in Prespa-Ohrid Watershed. Ph.D. Thesis, Aristotle University of Thessaloniki-School of Geology, Thessaloniki, Greece, 2021. [\[CrossRef\]](#)
47. Manolopoulos, A. *Estimation of Bathymetry and Stage to Volume Relations in Lake Mikri Prespa*; Internal Report; Society for the Protection of Prespa: Agios Germanos, Greece, 2017.
48. Hellenic Cadastre catalog, S.A. 2020. Digital Elevation Model (DEM). Available online: <https://data.ktimatologio.gr/> (accessed on 20 September 2024).
49. Demek, J.; Embleton, C. *Guide to Medium-Scale. Geomorphological Mapping*; E. Schweizerbart'sche Verlagsbuchhandlung, Nägele u. Obermiller: Stuttgart, Germany, 1978.
50. Roger, K.; Aminot, A. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. *Mar. Chem.* **1997**, *57*, 265–275. [\[CrossRef\]](#)
51. Boltz, D.F.; Mellon, M.G. Spectrophotometric determination of phosphate as molybdiphosphoric acid. *Anal. Chem.* **1948**, *20*, 749–751. [\[CrossRef\]](#)

52. APHA/AWWA/WPCF. *Standard Methods for the Examination of Water and Waste Water*, 15th ed.; Public Health Association/American Water Works Association/Water Pollution Control Federal: Washington, DC, USA, 1980; p. 1134.
53. US Environmental Protection Agency. *Methods for Chemical Analysis of Water and Wastes, Method 353.3*; USEPA Report No. EPA/600/4-79/020; Environmental Monitoring and Support Lab, Office of Research and Development: Washington, DC, USA, 1983.
54. Navone, R. Proposed method for nitrate in potable waters. *J.-Am. Water Works Assoc.* **1964**, *56*, 781. [[CrossRef](#)]
55. Pujo-Pay, M.; Raimbault, P. Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar. Ecol. Prog. Ser.* **1994**, *105*, 203–207. [[CrossRef](#)]
56. Raimbault, P.; Pouvesle, W.; Diaz, F.; Garcia, N.; Sempere, R. Wet oxidation and automated colorimetry for simultaneous determination of organic carbon, nitrogen and phosphorus dissolved in seawater. *Mar. Chem.* **1999**, *66*, 161–169. [[CrossRef](#)]
57. Skoulikidis, N.; Amaxidis, Y.; Bertahas, I.; Laschou, S.; Gritzalis, K. Analysis of factors driving stream water composition and synthesis of management tools—A case study on small/medium Greek catchments. *Sci. Total Environ.* **2006**, *362*, 205–241. [[CrossRef](#)] [[PubMed](#)]
58. Cardoso, A.C.; Duchemin, J.; Magoarou, P.; Premazzi, G. *Criteria for the Identification of Freshwaters Subject to Eutrophication*; EUR 19810 EN; EC Joint Research Centre: Ispra, Italy, 2001.
59. *EN 13946*; Guidance Standard for the Routine Sampling and Pretreatment of Benthic Diatoms from Rivers. European Committee for Standardization: Brussels, Belgium, 2003; CEN/TC 230. 14p.
60. *EN 14407*; Guidance Standard for the Identification. Enumeration and Interpretation of Benthic Diatom Samples from running Waters. European Committee for Standardization: Brussels, Belgium, 2004; CEN/TC 230. 12p.
61. Cantonati, M.; Kelly, M.G.; Lange-Bertalot, H. *Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assessment*; Koeltz Botanical Books: Schmittgen-Oberreifenberg, Germany, 2017; 942p, ISBN 978-3-946583-06-6.
62. Cemagref. *Etude des Méthodes Biologiques D'appréciation Quantitative de la Qualité des Eaux*; Technical Report; Lyon-Agence De l'eau Rhone-Méditerranée-Corse: Lyon, France, 1982; 218p.
63. Lazaridou, M.; Ntislidou, C.; Karaouzas, I.; Skoulikidis, N. Harmonisation of a new assessment method for estimating the ecological quality status of Greek running waters. *Ecol. Indic.* **2018**, *84*, 683–694. [[CrossRef](#)]
64. Søndergaard, M.; Jeppesen, E.; Jensen, J.; Amsinck, P. Water Framework Directive: Ecological classification of Danish lakes. *J. Appl. Ecol.* **2005**, *42*, 616–629. [[CrossRef](#)]
65. OECD; Vollenweider, R.A.; Kerekes, J.J. Eutrophication of waters. In *Monitoring, Assessment, and Control*; OECD: Paris, France, 1982; 192p.
66. EPA—US Environmental Protection Agency. *Nutrient Criteria, Technical Guidance Manual, Lakes and Reservoirs*, 1st ed.; EPA-822-B00-001; EPA—US Environmental Protection Agency: Washington, DC, USA, 2000.
67. Carlson Robert, E. A trophic state index for lakes. *Limnol. Oceanogr.* **1977**, *22*, 361–369. [[CrossRef](#)]
68. Meyer, M.F.; Kraemer, B.M.; Barbosa, C.C.; Cunha, D.G.F.; Dodds, W.K.; Hampton, S.E.; Ordóñez, C.; Pilla, R.M.; Pollard, A.I.; Culpepper, J.A.; et al. Clarifying the Trophic State Concept to Advance Macroscale Freshwater Science and Management. *Ecosphere* **2025**, *16*, e70392. [[CrossRef](#)]
69. Izabela, Z.; Jarosław, J.; Monika, R.; Michał, W. Relative impact of environmental variables on the lake trophic state highlights the complexity of eutrophication controls. *J. Environ. Manag.* **2023**, *345*, 118679. [[CrossRef](#)] [[PubMed](#)]
70. Søndergaard, M.; Johansson, L.S.; Levi, E.E.; Lauridsen, T.L.; Jeppesen, E. Lake types and their definition: A case study from Denmark. *Inland Waters* **2020**, *10*, 227–240. [[CrossRef](#)]
71. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; R Core Team: Vienna, Austria, 2019. Available online: <https://www.R-project.org/> (accessed on 20 October 2025).
72. Wickham, H.; Chang, W.; Henry, L.; Pedersen, T.L.; Takahashi, K.; Wilke, C.; Woo, K.; Yutani, H.; Dunnington, D. Package 'ggplot2': Create Elegant Data Visualisations Using the Grammar of Graphics; R package version 3.3.3 2020. Available online: <https://cran.r-project.org/package=ggplot2> (accessed on 20 October 2025).
73. Kassambara, A. ggpubr: 'ggplot2' Based Publication Ready Plots. R Package Version 0.5.0. 2020. Available online: <https://CRAN.Rproject.org/package=ggpubr> (accessed on 20 October 2025).
74. Clarke, K.R.; Gorley, R.N. *PRIMER v7: User Manual/Tutorial*; PRIMER-e: Auckland, New Zealand, 2015.
75. Clarke, K.R.; Gorley, R.N.; Somerfield, P.J.; Warwick, R.M. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 3rd ed.; PRIMER-e: Auckland, New Zealand, 2014.
76. Penman, H.L. *Natural Evaporation from Open Water, Bare Soil, and Grass*; Proceedings; Royal Society: London, UK, 1948; Volume 193, pp. 120–146.
77. Parissopoulos, G. *Review of Secchi Disk Measurements of Lesser Prespa Lake*; Technical Report; Society for the Protection of Prespa: Laimos, Greece, 2015; 12p.
78. Society for the Protection of Prespa (SPP). Secchi Disk Data Provision. Available online: <https://spp.gr/> (accessed on 20 July 2024).

79. Ministry of the Environment and Energy, Special Secretariat for Water, 2014. Approved Management Plan (Government Gazette): Approval of the River Basin Management Plan for the Western Macedonia Water District (GR09). Available online: https://wfdver.ypeka.gr/wp-content/uploads/2017/04/FEK_181.B.2014_GR09-1.pdf (accessed on 25 July 2025).
80. Ministry of the Environment and Energy, Special Secretariat for Water. 1st Revision of the River Basin Management Plan for the Western Macedonia Water District (EL09). 2017. Available online: https://wfdver.ypeka.gr/wpcontent/uploads/2021/02/EL09_1REV_P06_Tipo_Sinthikes_Anaforas.pdf (accessed on 20 June 2025).
81. Ministry of the Environment and Energy, Special Secretariat for Water. 2nd Revision of the River Basin Management Plans (RBMPs) for the Western Macedonia Water District (EL09). 2024. Available online: https://wfdver.ypeka.gr/wp-content/uploads/2024/07/EL09_2REV_sdlap.pdf (accessed on 20 June 2025).
82. UNDP-GEF Project. Technical Task Team (TTT) for the collection, assessment and evaluation of national information in support of the Transboundary Diagnostic Analysis (TDA) and development of a Strategic Action Programme (SAP) in the Prespa Lakes Basin. In *Transboundary Diagnostic Analysis (TDA)—Prespa Lakes Basin*; United Nations Development Programme (UNDP): New York, NY, USA; Global Environment Facility (GEF): Washington, DC, USA, 2009.
83. Heiskary, S.; Wilson, B. *Minnesota Lake Water Quality: Developing Nutrient Criteria*, 3rd ed.; Minnesota Pollution Control Agency: St. Paul, MN, USA, 2005.
84. Kuriata-Potasznik, A. The Functioning of a Water Body within a Fluvio- Lacustrine System as an Effect of Excessive Nitrogen Loading-The Case of Lake Symsar and its Drainage Area (Northeastern Poland). *Water* **2018**, *10*, 1163. [[CrossRef](#)]
85. Saunders, D.L.; Kalff, J. Nitrogen Retention in Wetlands, Lakes and Rivers. *Hydrobiologia* **2001**, *443*, 205–212. [[CrossRef](#)]
86. Sun, X.; Bernard-Jannin, L.; Grusson, Y.; Sauvage, S.; Arnold, J.; Srinivasan, R.; Sánchez Pérez, J.M. Using SWAT-LUD Model to Estimate the Influence of Water Exchange and Shallow Aquifer Denitrification on Water and Nitrate Flux. *Water* **2018**, *10*, 528. [[CrossRef](#)]
87. Uuemaa, E.; Palliser, C.; Hughes, A.; Tanner, C. Effectiveness of a Natural Headwater Wetland for Reducing Agricultural Nitrogen Loads. *Water* **2018**, *10*, 287. [[CrossRef](#)]
88. KfW Feasibility Study, GFA Consulting Group. *Project Preparation & Development of the Transboundary Prespa Park Project: Part V Hydrology Report*; KfW Entwicklungsbank: Frankfurt, Germany, 2005; 94p.
89. Popov, V.; Anovska, E.; Arsov, M.; Amataj, S.; Kolaneci, M.; Stamos, A.; Arsov, L.; Anovski, T.; Kiri, E.; Gelaj, A. Study of Prespa-Ohrid lake system using tracer experiments and the lake's water balance. *WIT Trans. Eng. Sci.* **2009**, *125*, 75–84.
90. Van der Schriek, T.; Giannakopoulos, C. Determining the causes for the dramatic recent fall of Lake Prespa (southwest Balkans). *Hydrol. Sci. J.* **2017**, *62*, 1131–1148. [[CrossRef](#)]
91. Eftimi, R.; Skende, P.; Zoto, J. Isotope study of the connection of Ohrid and Prespa lakes. *Geol. Balc.* **2002**, *32*, 43–50. [[CrossRef](#)]
92. Nelson, D.B.; Basler, D.; Kahmen, A. Precipitation isotope time series predictions from machine learning applied in Europe. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2024107118. [[CrossRef](#)]
93. Chen, M.; Li, X.; de Klein, J.; Janssen, A.B.G.; Du, X.; Lei, Q.; Liu, H.; Kroeze, C. Long-Term Responses of Internal Environment Dynamics in a Freshwater Lake to Variations in External Nutrient Inputs: A Model Simulation Approach. *Sci. Total Environ.* **2024**, *951*, 175514. [[CrossRef](#)]

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