*Article*

# Water Mixing Conditions Influence Sentinel-2 Monitoring of Chlorophyll Content in Monomictic Lakes

Michela Perrone [1,2], Massimiliano Scalici [2,*], Luisa Conti [1], David Moravec [1], Jan Kropáček [3], Maria Sighicelli [4], Francesca Lecce [4] and Marco Malavasi [1]

1   Department of Spatial Sciences, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha Suchdol, Czech Republic; perrone@fzp.czu.cz (M.P.); conti@fzp.czu.cz (L.C.); dmoravec@fzp.czu.cz (D.M.); malavasi@fzp.czu.cz (M.M.)
2   Department of Sciences, University Roma Tre, Viale Guglielmo Marconi 446, 00146 Rome, Italy
3   Department of Physical Geography and Geoecology, Faculty of Science, Charles University, Albertov 6, 128 00 Praha, Czech Republic; jkropacek@fzp.czu.cz
4   Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) CR Casaccia, 00123 Rome, Italy; maria.sighicelli@enea.it (M.S.); francesca.lecce@enea.it (F.L.)
*   Correspondence: massimiliano.scalici@uniroma3.it

**Abstract:** Prompt estimation of phytoplankton biomass is critical in determining the ecological quality of freshwaters. Remote Sensing (RS) may provide new opportunities to integrate with situ traditional monitoring techniques. Nonetheless, wide regional and temporal variability in freshwater optical constituents makes it difficult to design universally applicable RS protocols. Here, we assessed the potential of two neural networks-based models, namely the Case 2 Regional CoastColour (C2RCC) processor and the Mixture Density Network (MDN), applied to MSI Sentinel-2 data for monitoring Chlorophyll (Chl) content in three monomictic volcanic lakes while accounting for the effect of their specific water circulation pattern on the remotely-sensed and in situ data relation. Linear mixed models were used to test the relationship between the remote sensing indices calculated through C2RCC ($I_{NN}$) and MDN ($I_{MDN}$), and in situ Chl concentration. Both indices proved to explain a large portion of the variability in the field data and exhibited a positive and significant relationship between Chl concentration and satellite data, but only during the mixing phase. The significant effect of the water circulation period can be explained by the low responsiveness of the RS approaches applied here to the low phytoplankton biomass, typical of the stratification phase. Sentinel-2 data proved their valuable potential for the remote sensing of phytoplankton in small inland water bodies, otherwise challenging with previous sensors. However, caution should be taken, since the applicability of such an approach on certain water bodies may depend on hydrological and ecological parameters (e.g., thermal stratification and seasonal nutrient availability) potentially altering RS chlorophyll detection by neural networks-based models, despite their alleged global validity.

**Keywords:** remote sensing; mediterranean area; Phyto-PAM; volcanic lake; phytoplankton; water management; C2RCC; MDN; water framework directive; water quality

## 1. Introduction

It is widely shared by scientists and public opinion that freshwater ecosystems are of high importance for human wellbeing, although diverse activities act as environmental stressors detrimentally impacting the aquatic habitat quality, functionality, and status [1]. Major pressures such as nutrient pollution, deforestation, and urbanization are strongly reducing water quality, leading to the degradation of freshwater habitats [2–4]. In the EU territory, preserving and monitoring water quality has raised growing concern, leading to the adoption of the Water Framework Directive (Directive 2000/60/EC or WFD). WFD encourages policymakers, environmental managers, and researchers to plan and adopt yearly monitoring programmes to improve the ecological quality of all waters within the

territory. Phytoplankton biomass monitoring is a conventional method, also included in the WFD guidelines, for assessing water quality status in both inland [5] and marine waters [6]. Specifically, since chlorophyll-a (Chl-a) is the main pigment in photosynthetic organisms, it represents a suitable proxy for phytoplankton biomass estimation [7], thus relating to nutrient concentration and to freshwater trophic state.

However, the need for consistent, long-term assessment of freshwater ecological status urges integrative, frequent, and synoptic approaches. While in situ sampling of photosynthetic pigments is expensive and labour-intensive, as well as spatially and temporally limited, Remote Sensing (RS) provides new opportunities to integrate and improve traditional monitoring techniques [8–10]. Further, the increasing number of orbiting satellites makes remote sensing a cost-effective, energy- and time-saving method without equal. In particular, the recent launch of high spatial resolution satellites providing free and open data (e.g., Landsat-8 and Sentinel-2) has enhanced remote freshwater investigations, resulting in an ever-growing number of studies [11–15] with the specific features of the MultiSpectral Instrument (MSI) onboard Sentinel-2 satellites offering additional advantages for water quality measurement [16]. The MSI sensor measures Earth's reflected radiance in 13 spectral bands. Given its high spatial resolution (10–60 m) in the visible region, and its 12-bit radiometric resolution, MSI allows fine-scale measurement of water properties, enabling the monitoring of small water bodies [13,17]. Furthermore, Sentinel-2 temporal resolution of 2–5 days provides frequent data acquisition, allowing constraints to be overcome (such as frequent cloud cover) and to detect rapid changes of phytoplankton biomass [18].

Remote sensing-based detection of Chl relies on the application of specific indices depending on the biogeochemical characteristics of the water body, which leads to the distinction between case-I and case-II waters [19,20]. The former are waters where phytoplankton and its degradation products dominate optical properties (e.g., open oceans), while inland waters are categorised as case-II waters, being subject to potentially large and independent variations of Chl and other optically active suspended substances. Given the wide regional and temporal variability in constituents of case-II waters, a large number of Chl-retrieving algorithms have been developed and tested so far (e.g., semi-empirical methods or semi-analytical approaches) [15,17,21–29], making the selection of the most suitable index sometimes intricate [27,30,31]. Indeed, specific algorithms are often optimized and validated for commonly understood but not always straightforward to define water types, e.g., turbid [32] or clear water [33]. Other statistical methods like neural networks (NN) based on spectral inversion algorithms for radiative transfer simulations [34] have successfully been applied, is intended for use under different bio-optical regimes and without a priori knowledge of the in-water optical conditions (being trained to varying parameter concentration and optical property ranges), sometimes outperforming other methods [27,31,35–38].

Among these, the Case-2 Regional CoastColour processor (C2RCC) [39] is a "user-friendly" method using MSI data that is readily available in the ESA's SentiNel Application Platform (SNAP) [28]. C2RCC functioning relies on a set of neural networks deriving water inherent optical properties, which are then converted into Chl-a and total suspended matter (TSM) concentrations. The C2RCC processor is an improvement of the Case 2 Regional processor originally developed by Doerffer and Schiller [34], integrating an atmospheric correction part dedicated for water observation, and has been trained to cover even extreme ranges of scattering and absorption [39]. On the other hand, the Mixture Density Network (MDN) is a recently developed algorithm by Pahlevan et al. [27], which is a variation of Multilayer Perceptrons NN methods and relies on a class of neural networks that estimates multimodal Gaussian distributions over a range of solutions. The MDN algorithm has been trained with 1000 co-located in situ $R_{rs}$- Chl-a pairs and was initially tested with Sentinel-2 and Sentinel-3 data, outperforming previously published algorithms (e.g., OC and Blend) [27]. Still, both C2RCC and MDN are far from being considered universally

applicable approaches for Chl retrieval, and their efficiency needs to be tested under different conditions.

Water quality analysis of volcanic lakes has rarely been addressed by applying RS techniques [40,41] and, as far as we know, none of these studies has investigated warm monomictic volcanic lakes using MSI data. In such lakes, temperatures never drop below 4 °C and thermal stratification occurs during summer, when air temperature increases, and surface water is heated more rapidly. Water circulation occurs only once a year when surface waters cool to a temperature equal to the bottom waters. In most temperate and sub-tropical warm monomictic lakes, water column mixing brings hypolimnetic nutrients to the surface, leading to annual maximum phytoplankton proliferation [42–44]. Such a marked variation in primary production between the thermally stratified season and the full water column mixing could define a sharp temporal divergence in the viability of RS observation of such water bodies.

Accordingly, the purpose of this paper is to assess the potential of two open-source algorithms (i.e., C2RCC and MDN) applied to MSI Sentinel-2 data to retrieve Chl content in three monomictic volcanic lakes (Central Italy) while accounting for their water circulation pattern. Given the appropriateness of both processors for a broad range of bio-optical conditions, we hypothesize that the water circulation pattern of such lakes (i.e., one mixing and one stratification phase) will not affect the strength of the relationship between remote sensing and field data. Due to growing anthropogenic activity and to an increasing frequency of algal blooms, the need for trophic level assessment of Italian volcanic lakes has become essential for their preservation [45].

## 2. Materials and Methods

### 2.1. Study Area

This study was carried out in three warm monomictic volcanic lakes located in the Latium region, Italy: Lake Albano, Lake Bracciano, and Lake Nemi (Figure 1).
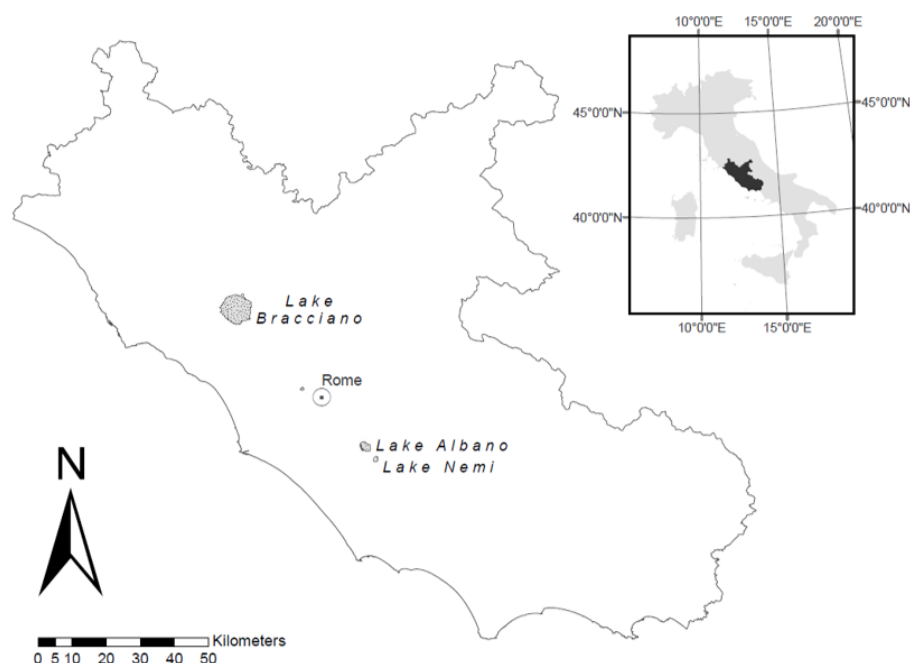


**Figure 1.** Location of the three investigated volcanic lakes within the study area. Datum: WGS 84, UTM 32N.

These lakes are all located in craters originated from Quaternary volcanic activity [46], which determines their circular shape and great depth compared to the relatively low surface area [44] (further described in Appendix A). As their water input is nearly exclusively provided by underground springs and rainfall, these lakes are characterized by long water

renewal times, which can represent an additional risk factor if deterioration phenomena occur [45,47].

Based on the temperature data of the water column collected by the Regional Agency for Environmental Protection of Lazio (ARPA Lazio), in all three lakes, the seasonal thermal stratification for the year 2019 started in spring, in agreement with findings from previous studies reporting similar trends [42,45]. According to the water circulation phases, chlorophyll concentration data were grouped as follows (Figure 2): mixing from November to April, stratification from May to October.
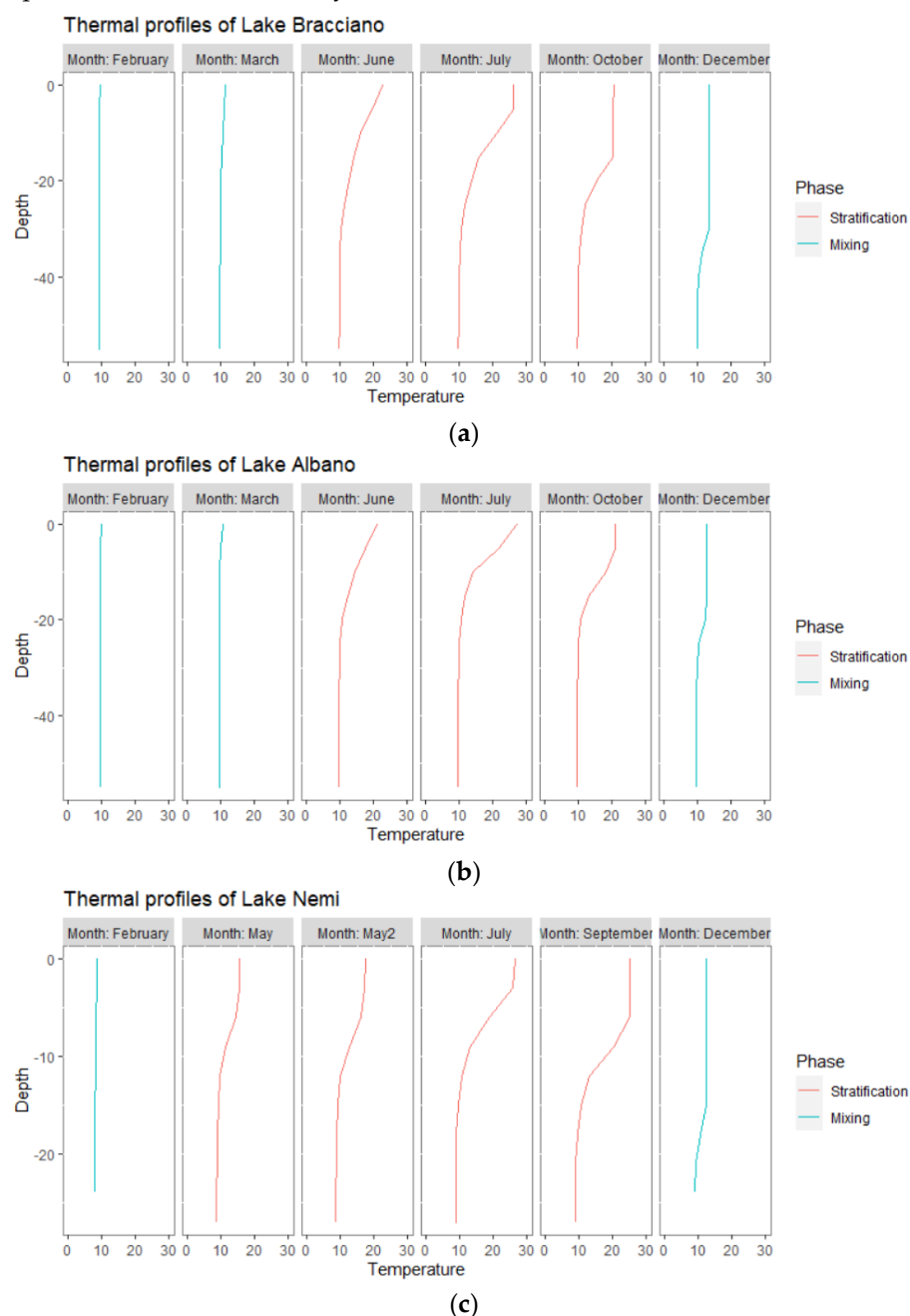


**Figure 2.** Water thermal profiles in 2019 of: (**a**) Lake Bracciano, (**b**) Lake Albano, and (**c**) Lake Nemi (data from two sampling dates in the month of May are available: May = 6 May 2019 and May2 = 31 May 2019). Blue lines represent temperature profiles of the mixing phase, during which little or no differences between the surface and deep layers of the water column were observed. Red lines represent thermal stratification, where the water column is divided into epilimnion (top, warmer layer), metalimnion (with rapid temperature change), and hypolimnion (bottom, colder layer).

### 2.2. Digital Data Collection and Processing

Sentinel-2 MSI L1C products were downloaded from the Copernicus Open Access Hub (https://scihub.copernicus.eu (accessed on 28 October 2019)). L1C products contain orthorectified, georeferenced Top of Atmosphere (TOA) reflectance in UTM map projection with WGS84 datum. Matchups with in situ measurements were selected with a mean time difference between suitable Sentinel-2 and in situ data of ~2 days. For only one matchup a maximum time difference of 8 days with Sentinel-2 MSI overpass was allowed (Table 1), provided that weather conditions were stable between the image acquisition and field sampling and that no algal blooms occurred. This time window was set to allow sufficient matchups between ground data and Sentinel-2 imagery.

**Table 1.** Sentinel-2 image acquisition dates and sampling dates ("-" indicates the absence of suitable satellite data).

| Lake Albano | | | Lake Bracciano | | | Lake Nemi | | |
|---|---|---|---|---|---|---|---|---|
| Sampling Date | Image Acquisition Date | Time Difference (d) | Sampling Date | Image Acquisition Date | Time Difference (d) | Sampling Date | Image Acquisition Date | Time Difference (d) |
| 19 March 2019 | 22 March 2019 | 3 | 18 March 2019 | 22 March 2019 | 4 | 19 March 2019 | 22 March 2019 | 3 |
| 2 April 2019 | 1 April 2019 | 1 | 1 April 2019 | 30 March 2019 | 2 | 2 April 2019 | 1 April 2019 | 1 |
| 16 April 2019 | - | - | 17 April 2019 | 19 April 2019 | 2 | 16 April 2019 | - | - |
| 16 May 2019 | - | - | | | | 16 May 2019 | - | - |
| 28 May 2019 | 5 June 2019 | 8 | 30 May 2019 | 31 May 2019 | 1 | 29 May 2019 | 5 June 2019 | 7 |
| 12 June 2019 | 15 June 2019 | 3 | 10 June 2019 | 13 June 2019 | 3 | 12 June 2019 | 15 June 2019 | 3 |
| 27 June 2019 | 25 June 2019 | 2 | 26 June 2019 | 25 June 2019 | 1 | 27 June 2019 | 25 June 2019 | 2 |
| 9 July 2019 | 5 July 2019 | 4 | | | | 9 July 2019 | 5 July 2019 | 4 |
| 23 July 2019 | 25 July 2019 | 2 | 25 July 2019 | 25 July 2019 | 0 | 23 July 2019 | 25 July 2019 | 2 |
| 5 September 2019 | 3 September 2019 | 2 | 17 September 2019 | 18 September 2019 | 1 | 5 September 2019 | 3 September 2019 | 2 |
| 21 October 2019 | 23 October 2019 | 2 | | | | 21 October 2019 | 23 October 2019 | 2 |

Satellite images were visually checked to make sure that the studied lakes were not covered by clouds or cloud shadows before application by exploiting the spectral band at 1375 nm (band 10), which enables cirrus detection. Due to cloud cover on images, no satellite data are available for two sampling dates for Lake Albano and Lake Nemi.

Two different indices were implemented, one calculated through the neural networks of the C2RCC processor ($I_{NN}$) and one calculated through the MDN algorithm ($I_{MDN}$). The Case 2 Regional Coast Colour (C2RCC) processor (v1.0) is readily available on SNAP among the Thematic Water Processing tools. Getting as input the water leaving reflectances from the atmosphere part, the in-water part performs the inversion of the spectrum into inherent optical properties (IOPs), i.e., absorption and backscattering coefficients, from which Chl and total suspended matter concentrations are calculated. The simulations on which the C2RCC is built are based on a large set of optical data and concentration measurements, enabling the processor to cover extreme ranges of scattering and absorption and making it suitable for a large variety of study areas [34]. To better adapt the processing to the conditions of the study area, C2RCC allows flexibility in adjusting ancillary parameters. Among the processing parameters, we adjusted elevation, salinity, and temperature according to local values registered during each sampling campaign. The CHL exponent and the CHL factor required to better estimate Chl concentration remained unchanged from the default values, as it was not possible to retrieve such parameters from the lakes under study. To test the suitability of this approximation, we assessed the relationship between in situ measured Chl and Chl absorption coefficient (iop_apig) calculated by the C2RCC (the results are presented in Appendix B).

The MDN algorithm was recently developed by Pahlevan et al. [27] and is freely available on GitHub (https://github.com/STREAM-RS/STREAM-RS (accessed on 26 May 2021)). Such processor models conditional probability distributions of the target variables (i.e., Chl-a) as a mixture of multiple Gaussian functions from remote sensing reflectance ($R_{rs}$). The Gaussians are subsequently combined to form the final output estimation through maximum likelihood [48]. In the case of the MSI sensor, the processor requires as input $R_{rs}$ values at the following wavelengths: 443 nm, 490 nm, 560 nm, 665 nm, 705 nm, 740 nm,

and 783 nm (i.e., Band 1 through Band 7). In the present study, $R_{rs}$ reflectance values were calculated via the C2RCC atmospheric correction.

The SNAP Pixel Extraction tool was later used to extract pixel values from the GeoTIFF products obtained from both types of processing. To minimize errors due to boat drift and GPS positional inaccuracies, the median value of a $3 \times 3$ pixel window centred at each sampling point was extracted and used for further statistical analysis.

### 2.3. In Situ Data

To validate remote sensing data, a total of 30 sampling campaigns were conducted over the period March-October 2019. Samples were collected at five sampling sites distributed uniformly across each lake. The sampling site coordinates were recorded, and geolocation uncertainties related to boat drift and GPS positional inaccuracies were taken into account in the satellite data analysis ($3 \times 3$ pixel windows, see Section 2.2). Concurrently, epilimnetic water temperature, pH, salinity, and dissolved oxygen (DO) concentration were recorded at each sampling site using a Hach HQ40d Portable Multi-Parameter Meter.

To estimate water Chl content, 2 L water samples, one at each sampling site, were collected from the surface layer (max. 50 cm depth, according to previous literature) with a Van Dorn water sampler [13,21,26–29,49]. Samples were stored on ice in sterile, light-reflective containers (to prevent pigment degradation).

Within 24 h of collection, water samples underwent laboratory measurement of their Chl content. In this study, total Chl concentration measurements were carried out using the Phyto-PAM—Multiple Excitation Wavelength Phytoplankton & Photosynthesis Analyzer (Heinz Walz GmbH, Effeltrich, Germany). This instrument allows non-invasive and rapid biomass estimates since it does not require sample treatments before carrying out the analysis. The functioning of this fluorimeter relies on the selective amplification of a fluorescence signal, measured with the help of short (µsec) pulses of measuring light at a high repetition rate (1200 Hz). These light pulses are provided by an array of light-emitting diodes at four distinct light wavelengths (470, 520, 645, and 665 nm). A photomultiplier in conjunction with a pulse amplifier is used as a detector for fluorescence at wavelengths above 710 nm. The information consisting of four independent fluorescence signals is further processed by computer-controlled data analysis using the dedicated PhytoWin-software. Three measurements were carried out for each water sample. A Zero offset (Zoff) determination preceded fluorescence measurement to remove other fluorescing substances' contribution to the measured signal (e.g., humic acids).

As remote sensing indices are developed to retrieve Chl-a concentration and the Phyto-PAM instrument measures total Chl concentration, a standard spectrophotometric method was applied to a representative subset of water samples to validate the use of the Phyto-PAM results (further description of the validation process can be found in Appendix C). We assumed that Phyto-PAM's concentration values were consistent with Chl-a concentration. Water samples were filtered through Whatman GF/F glass microfiber filters (0.7 µm pore size) under vacuum pressure. Filters were subsequently put in 5 mL of 90% acetone solution and then homogenised, and acetone solution was added to the emulsion to reach the final volume of 10 mL. Sample extracts were stored at 4 °C in the dark for 24 h for pigment extraction. Extracts were clarified by centrifugation (3500 rpm, 12 min) and then transferred to a 15-mL vial to measure their final volumes. Triplicate absorbance measurements were carried out on each water sample using a dual-beam spectrophotometer. The resulting absorbance values at 630 nm, 647 nm, 664 nm, and 750 nm were entered into the equation by Jeffrey & Humphrey (1975) [50] to estimate Chl-a concentration.

### 2.4. Statistical Analysis

To test the relationship between remote sensing derived indices and in situ Chl concentration, we assumed a positive linear relationship between the two sets of data. Due to the spatial interdependence of the samples in our dataset, we used linear mixed models (LMM) as they allowed us to use the lake identity as a random intercept effect. We used R software v. 3.6.1 [51] and lme4 package [52] to fit the linear mixed-effect model, using the indices as the dependent variable and the in situ Chl content as a fixed-effect independent variable. Further, we considered the interaction of in situ Chl concentration with the water mixing period (i.e., mixing from November to April, stratification from May to October), deleting the intercept to see the effect of the interaction. Finally, to determine the amount of variance in our in situ data explained by the models, we considered the model's conditional $R^2$ ($R^2c$), related to the variance explained by both fixed and random effects, and marginal $R^2$ ($R^2m$), related to the variance explained only by fixed effects [53]. No evident deviations from normality emerged after visual inspection of residual plots.

### 3. Results

### 3.1. In Situ Data

Descriptive statistical results of measured parameters are shown in Table 2. Over the sampling period, Chl and Chl-a concentrations exhibited comparable trends in all three lakes, with the highest values recorded in early spring and minimum values in July (Table 2). Epilimnetic water temperatures showed their lowest values in March and maximum values in July, followed by a slow decrease. Comparable values of DO and pH were observed among the three lakes, whereas salinity was significantly lower in lake Nemi compared to the other two lakes.

**Table 2.** Statistical description (mean, minimum, maximum, and standard deviation) of water properties: total chlorophyll concentration (Chl) measured spectrophotometrically, chlorophyll-a concentration (Chl-a) measured spectrophotometrically, epilimnetic water temperature (Temp), salinity (Sal), pH, dissolved oxygen concentration (DO) in the studied lakes.

| Lake | Chl (mg/L) | | | | Chl-a (mg/L) | | | | Temp (°C) | | | |
|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | Mean | Min | Max | SD | Mean | Min | Max | SD | Mean | Min | Max | SD |
| **Bracciano** | 1.68 | 0.44 | 3.73 | 0.95 | 1.29 | 0.44 | 2.51 | 0.60 | 18.37 | 10.47 | 26.47 | 6.03 |
| **Albano** | 3.69 | 0.42 | 20.2 | 4.56 | 3.17 | 0.33 | 19.1 | 4.2 | 21.1 | 11.1 | 30.1 | 6.3 |
| **Nemi** | 2.15 | 0.42 | 10.86 | 2.81 | 1.87 | 0.33 | 8.55 | 2.5 | 20.4 | 10.3 | 28.7 | 6.1 |
| **Lake** | **Sal (‰)** | | | | **PH** | | | | **DO (mg/L)** | | | |
| | Mean | Min | Max | SD | Mean | Min | Max | SD | Mean | Min | Max | SD |
| **Bracciano** | 0.27 | 0.26 | 0.27 | 0.01 | 8.31 | 7.14 | 8.92 | 0.37 | 9.57 | 8.3 | 10.38 | 0.79 |
| **Albano** | 0.24 | 0.22 | 0.25 | 0.01 | 8.42 | 8.04 | 8.65 | 0.15 | 0.27 | 8.57 | 10.68 | 8.42 |
| **Nemi** | 0.16 | 0.15 | 0.16 | 0.00 | 8.30 | 7.36 | 8.69 | 0.27 | 8.96 | 7.59 | 10.31 | 0.94 |

### 3.2. Indices Performance Assessment

When analysing the results for the $I_{NN}$ and $I_{MDN}$ models, we found significant dependency on the water mixing period. In particular, the relationship between $I_{NN}$ and the in situ data was significant when considering data in the mixing phase, while there was no significant relationship during the stratification period (Figure 3a). For the mixing phase, there was a positive relationship between the two sets of data, while for the stratification phase the relationship was negative, although not significant (Figure 3b).
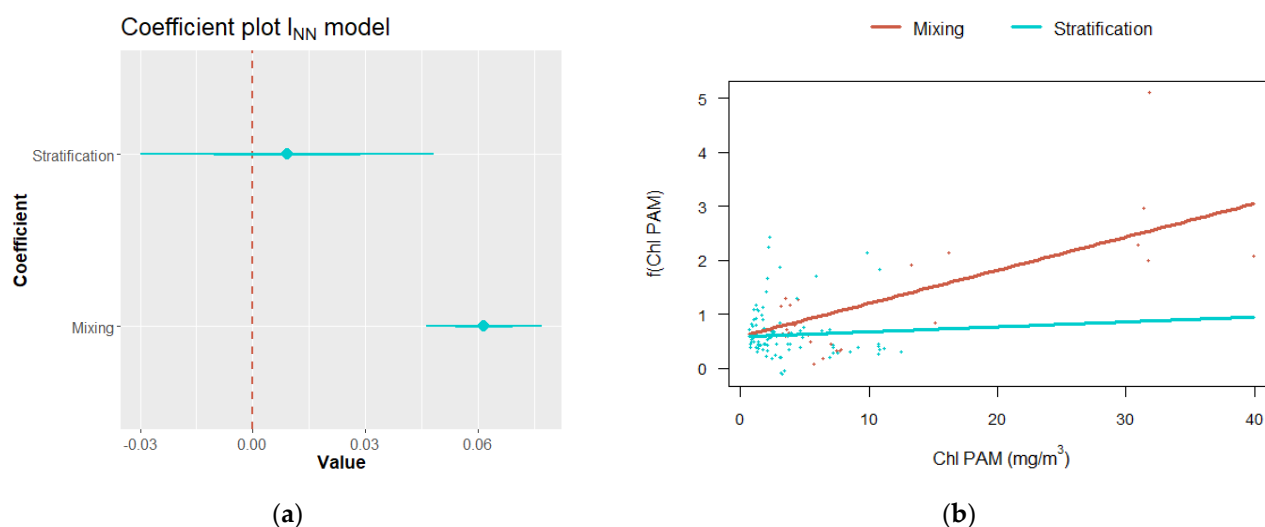
(a)                                                                    (b)

**Figure 3.** (**a**) Coefficient plot of the linear mixed model designed with the index based on the C2RCC ($I_{NN}$). Dots represent coefficient estimates; the outer error bars are 95% confidence intervals and the inner error bars are 50% confidence intervals. The 95% coefficient interval for stratification includes zero, which indicates a non-significant relationship between measured and predicted chlorophyll content. (**b**) Plot of the interaction effect between Chl concentration and water circulation phase.

With the $I_{MDN}$ index, similar outcomes were obtained in comparison to the model with $I_{NN}$. The relationship between Chl data and remote sensing data was positive and significant during the water mixing phase, whereas there was no significant relationship at low Chl concentrations, that is, during the stratification phase (Figure 4).

Furthermore, the variance explained by the two indices showed comparable values of $R^2m$ ($I_{NN}$ = 0.331, $I_{MDN}$ = 0.316) and $R^2c$ ($I_{NN}$ = 0.475, $I_{MDN}$ = 0.467), indicating that the neural networks-based approaches implemented in C2RCC and MDN are able to explain a satisfactory portion of the variability in the in situ data.
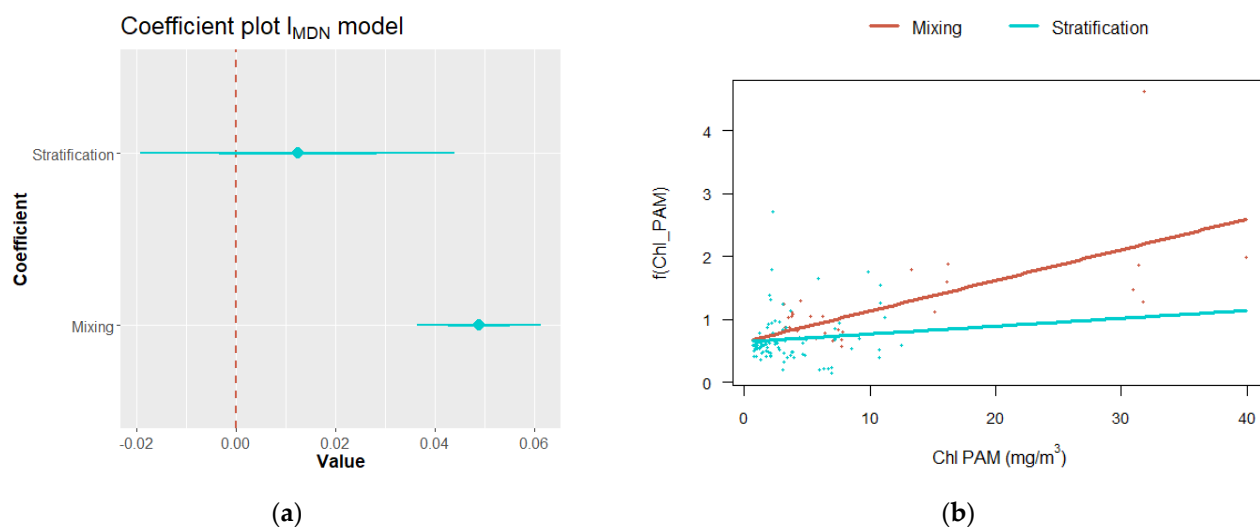


(a)                                                                    (b)

**Figure 4.** (**a**) Coefficient plot of the linear mixed model designed with the index based on the MDN algorithm ($I_{MDN}$). Dots represent coefficient estimates; the outer error bars are 95% confidence intervals and the inner error bars are 50% confidence intervals. The 95% coefficient interval for stratification includes zero, which indicates a non-significant relationship between measured and predicted chlorophyll content. (**b**) Plot of the interaction effect between Chl concentration and water circulation phase.

## 4. Discussion

The aim of this paper was to test the performance of the C2RCC and MDN processors applied to Sentinel-2 products for Chl concentration monitoring in three volcanic lakes of central Italy while assessing the influence of the water mixing conditions on the relationship between remote sensing and field data. We chose to apply (a) the C2RCC processor due to its ease of operation within the ESA's SentiNel Application Platform (SNAP) and wide applicability, thus potentially making it a favoured choice for many users; (b) the MDN algorithm as it is a recently developed method that proved to outperform previous, well-established methods when applied to Sentinel-2 data. Given the purpose of the present study, our efforts were focused on a restricted (but representative) number of comparable lakes over one seasonal cycle, unlike other studies investigating one [12,29] or several water bodies [15] on a single or limited dates.

This study showed that MSI data has potential for Chl estimation in monomictic volcanic lakes, proving Sentinel-2 satellites to be powerful tools for water quality monitoring. Indeed, the two models based on $I_{NN}$ and $I_{MDN}$ both exhibited a positive and significant relationship between Chl concentration and satellite data. Nonetheless, contrary to our expectations, such a significant relationship was observed only during the mixing phase, indicating that the water circulation pattern of such lakes (i.e., one mixing and one stratification phase) may affect the strength of the relationship between remote sensing and field data when using C2RCC and MDN processors.

Seemingly, the significant effect of thermal stratification on the performance of the RS-based index can be explained by the low responsiveness of the RS approach applied here to the low amount of phytoplankton biomass. Indeed, the large amount of nutrients made available by the full water circulation taking place during the mixing phase increases phytoplankton biomass, which can be more easily captured by RS techniques. Conversely, when the stratification phase is established, the water column is divided into three compartments: (1) an upper layer (epilimnion) of warm, oxygenated water, where the majority of primary production takes place; (2) a thin middle layer (metalimnion) where temperature changes more rapidly; and (3) a cold, low in dissolved oxygen layer (hypolimnion) where the decomposition of sedimenting organisms from the epilimnion occurs [43,44]. Hence, during thermal stratification, the organisms' metabolism within the epilimnion causes a progressive reduction of nutrient availability with a consequent phytoplankton mortality increase in this layer [54–58]. Accordingly, when considering data related to the stratification phase, the absence of a significant relationship between Chl concentration surveyed by the Phyto-PAM and the one retrieved by RS can have a threefold explanation.

First, several studies proved the important (and sometimes dominant) contribution that inorganic and organic sediments have on the optical properties of case-II waters [59–64]. Their effects on total backscattering are magnified when phytoplankton biomass is low. In this case, the remotely-sensed signal can be noticeably impacted by non-phytoplankton water constituents (e.g., minerals and CDOM) which are not correlated with Chl-a concentration [34,62,65,66]. In the case of spectral inversion algorithms, as in C2RCC, the ability of radiative transfer simulations to simultaneously solve several IOPs (i.e., absorption and backscattering coefficients) is sensitive to errors due to the existence of ambiguous solutions resulting from the additive nature of the IOPs. That is to say, different combinations of IOPs of each water component can lead to an identical sum of IOPs and, thus, to similar reflectance values [67,68]. On the contrary, the MDN algorithm attempts to overcome this one-to-many issue by modeling a conditional probability distribution of the target variables given input $R_{rs}$ data rather than directly modeling the $R_{rs}$- Chl-a relationship like standard NNs [27,48,69]. However, the results obtained in the present study show a comparable performance, indicating that this non-uniqueness problem is yet to be fully solved for the investigated lakes. Indeed, such ambiguity error on the total absorption coefficient is the highest for highly absorbing water bodies [67]. Therefore, even though the methods used in this study are suitable for phytoplankton monitoring, the difficulty of

sensing low Chl-a signals limits their effectiveness only to the mixing phase of the yearly water circulation pattern of this type of lakes. Moreover, the performance of this kind of approach is dependent on the ranges of the training parameters. Indeed, when the bio-optical properties of the investigated area are beyond training ranges, this is expected to adversely impact the NN efficiency of retrieving Chl concentration [70,71].

Second, the absence of a significant correspondence between remotely-sensed data and field data could be concurrently ascribed to the MSI sensor specifications in terms of Signal-to-Noise Ratio (SNR). Sentinel-2 MSI SNR (generally below 150:1 for most of its spectral bands) is much lower than the minimum SNR required for Chl retrievals in marine waters, which is set at 400:1 for all visible bands and at 600:1 for NIR bands [72,73]. However, improving SNR values when designing sensors requires trade-offs in terms of reduced spatial and spectral resolution, as well as other radiometric characteristics [72,74]. Due to the relatively low Sentinel-2 SNR, it is reasonable to assume that the MSI sensor mainly measures noise when Chl concentration is low. Consequently, a higher relative error in the sensed signal is expected [75], which affects the significance of the indices during the stratification phase.

## 5. Conclusions

Sentinel-2 data proved their valuable potential for remote sensing of phytoplankton in small inland water bodies, otherwise challenging with previous sensors. This study confirmed the feasibility of C2RCC and MDN applied to Sentinel 2 data to monitor chlorophyll content in warm monomictic lakes. The approach presented has a good potential for operational monitoring of small and medium-sized water bodies required, for instance, by the European Union Water Framework Directive. Nonetheless, it is worth highlighting the limits intrinsically embedded in our dataset. Indeed, this study was carried out on a group of peculiar lakes and over just one year, without an optimal (although acceptable) time match-up between ground data and Sentinel-2 imagery. Additionally, the absence of simultaneous field radiometry data did not allow an accurate validation of the atmospheric correction.

Caution should be taken when relying on remote sensing for water quality monitoring (i.e., C2RCC and MDN) because the consistency of such a method may depend on the hydrological and ecological processes associated with the water mixing conditions, which have not been addressed in the algorithm yet. Especially the low chlorophyll concentrations during the stratification phase can prove challenging for the performance of the tested approach, despite its claimed broad applicability. From the present study, it seems evident that preliminary ecological knowledge of the water body under investigation is of paramount importance for a proper application of the Chl-retrieving algorithms, even on a temporal scale. Therefore, indiscriminate use of the neural networks-based approaches assessed in this study without focusing on the local biological and hydrodynamic processes could lead to inconsistent results, because water thermal stratification of lakes could significantly affect retrieval.

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## Appendix A

Lake Bracciano is included in Bracciano-Martignano Regional Park and designated as a Site of Community Importance (SCI-IT6030010). Lake Bracciano has recently been used as a drinking water supply in the event of a city water emergency. The lake water crisis of 2017 caused by dry weather and constant uptake led to a substantial water-level reduction (up to −198 cm) and to water quality deterioration, causing growing concern for the preservation of the ecosystem integrity.

Lake Albano is ranked as meso-eutrophic [47,76], with progressive deterioration over time due to an increase of sewage discharge [77] and over-extraction of groundwater within the watershed. Cyanotoxic algal blooms regularly occur in Lake Albano, when the water column mixing brings hypolimnetic nutrients to the surface [76,78,79]. Even though its quality is gradually deteriorating, Lake Albano is designated SCI (IT6030038).

Together with Lake Albano, Lake Nemi is part of Castelli Romani Regional Park. Recent data reveal an improvement in Lake Nemi's water quality. After the implementation of domestic sewage diversion in the early 1990s, all biological and chemical data showed partial recovery, shifting Lake Nemi's trophic status from eutro-hypereutrophic to meso-eutrophic [46,47,77].

**Table A1.** Main features of the volcanic lakes under investigation.

|  | Lake Bracciano | Lake Albano | Lake Nemi |
|---|---|---|---|
| Location (Lat., Lon.) | 42°07′16″N 12°13′55″E | 41°45′0″N 12°39′54″E | 41°42′44″N 12°42′09″E |
| Max. depth (m) | 165 | 175 | 27.5 |
| Mean elevation (m a.s.l.) | 164 | 293 | 316 |
| Surface area (km$^2$) | 57.5 | 6.0 | 1.6 |
| Volume ($10^6$ m$^3$) | 5050 | 464 | 26.5 |
| Renewal time (yr) | 137 | 47.6 | 15 |
| Outflows | Arrone river (currently dry in its first stretch), Paul aqueduct | No natural outlets | No natural outlets |

## Appendix B

**Table A2.** Marginal R$^2$ (R$^2$m) and conditional R$^2$ (R$^2$c) of the linear mixed model based on the chlorophyll absorption coefficients retrieved from the C2RCC (iop_apig).

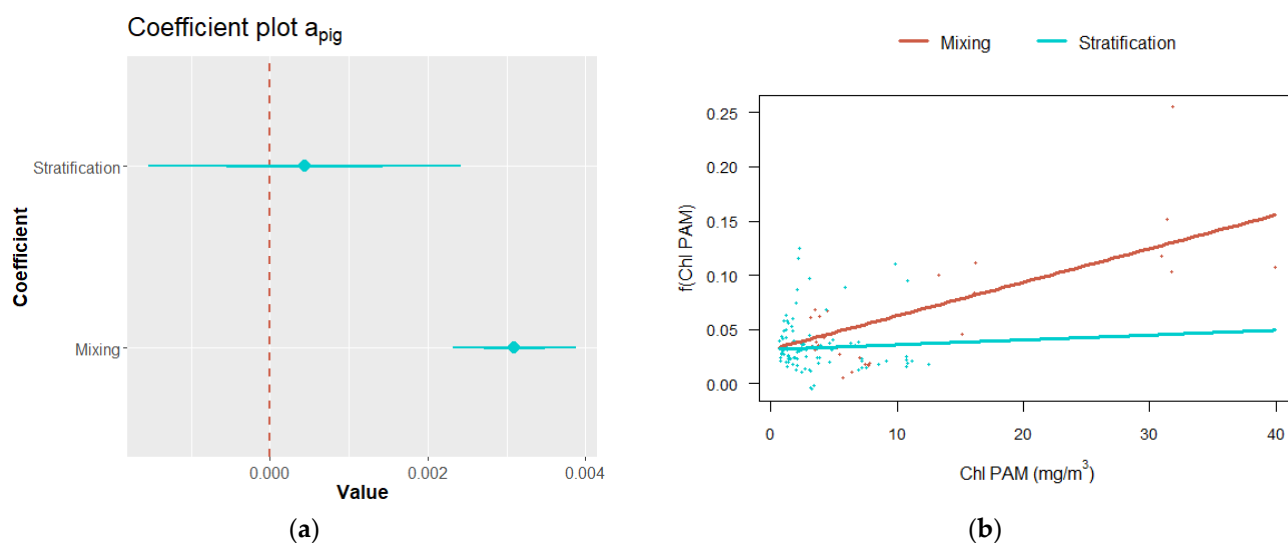| R$^2$m | R$^2$c |
|---|---|
| 0.326 | 0.476 |

**Figure A1.** (**a**) Coefficient plot of the linear mixed model designed with in situ measured Chl and Chl absorption coefficient ($a_{pig}$) calculated by the C2RCC. Dots represent coefficient estimates; the outer error bars are 95% confidence intervals and the inner error bars are 50% confidence intervals. The 95% coefficient interval for stratification includes zero, which indicates a non-significant relationship between measured chlorophyll content and pigment absorption coefficient estimates. (**b**) Plot of the interaction effect between $a_{pig}$ and water circulation phase.

## Appendix C

A correlation test between Phyto-PAM chlorophyll values and the ones obtained spectrophotometrically was performed. Before carrying out this test, it has been necessary to assess the effective relationship between total chlorophyll and chlorophyll-a, as well as testing whether this latter pigment was the major contributor to total Chl concentration. To this end, a T-test was used to calculate the differences between Chl-a and total chlorophyll concentration. The results indicate that Chl-a does not differ significantly from total chlorophyll ($p$-value = 0.402). The association between total Chl and Chl-a values was assessed using a correlation test, whose result was significant ($p$-value $< 2.22 \times 10^{-16}$), with $r = 0.99$. Based on the aforementioned results, total chlorophyll and Chl-a concentrations measured spectrophotometrically showed comparable values and trends. The result of the correlation test exhibits a significant and high association ($p$-value $< 2.22 \times 10^{-16}$, $r = 0.95$) between total Chl estimated by Phyto-PAM and through spectrophotometric measurements.

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