

## Article

# Abundance of Benthic Algae in Forestry Watersheds and the Associated Forest Cover Factors

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**Abstract:** We analyzed the abundance of benthic algae in nine forested headwater watersheds in south-central Chile to study the relationship between the variability of the abundance of benthic algae and physicochemical parameters and forest cover factors. Between 10 November 2015 and 18 August 2016, we sampled benthic algae and physicochemical parameters in the streams and characterized forest cover factors in each of the study watersheds. We found that physicochemical parameters and forest cover factors have contrasting effects on abundance. More specifically, the following observations were made: (1) maximum diversity and abundance occurred in watersheds covered with *Pinus radiata*, (2) seasonal ranks did not influence the abundance of benthic algae, (3) benthic algae abundance is controlled by rank dissimilarities in type of vegetation, solar radiation, volume, density age, canopy openness, native forest area, plantation area and harvested area, and (4) regression analysis of genera exhibited significant variability with physicochemical parameters (water temperature, pH, ammonium, nitrate, phosphorus, solar radiation) and forest cover factors (volume, density, age, native forest area, plantation area and harvested area); however, these effects were not seen in all genera. Factors related to the benthic algae compartment showed the highest sensitivity to forest cover, emphasizing their value as biological indicators of water quality and stream ecological integrity.

**Keywords:** benthic algae; physicochemical; forest cover; ranks dissimilarities



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## 1. Introduction

At a local scale, headwater stream systems, flow patterns, temperature and substrates appear to be the key variables that influence the conditions experienced by benthic algae [1]. Other more immediate impacts might be related to flow patterns (via terrain slope and roughness), temperature (via elevation and climate-dependent riparian vegetation), the geological environment and land use [2,3]. Additionally, the availability of light and the productivity of streams act as controlling factors for the composition of benthic algae at various riparian vegetation widths [4,5]. These conditions directly influence the streams' habitats and can be used to predict species composition [1]. Ref. [6] recognized the effect of light as a limiting factor for benthic primary productivity in riverine ecosystems. The composition of benthic algae can be related to water chemistry (pH, stream conductivity and nutrient concentrations), and factors such as substrate, light availability, and flow velocity [7–10].

The mid-latitude terrestrial ecosystems of the Chilean Coastal Range are characterized by a climate with dry summers and by highly productive forests of fast-growing exotic species (specifically *Pinus radiata* D. Don and *Eucalyptus globulus* Labill), which currently occupy 2.4 million hectares [11]. In these watersheds, stream benthic algae are strongly influenced by riparian vegetation through multiple forest cover processes acting on different

spatial and temporal scales. For example, discharge and solute and seston loads respond to rainfall and forest cover, soil, and geology physiography at the scale of the watershed, while stream hydraulics, light and organic inputs tend to be more sensitive to terrain slope, roughness, and the conditions of the riparian zone [1,12–14].

While the effects of intensive forest plantation on benthic algae seem clear in the northern hemisphere [15,16], the understanding of their impact on southern mid-latitude ecosystems such as those in Chile is far from complete, particularly when comparing different forest-management plantation systems. Numerous environmental evaluations of benthic algae communities have been used as stress or habitat-type indicators [17]. Studies of benthic algae in forests demonstrated that changes of algae composition were caused by increased light availability following harvest [18]. Shifts between species were partly explained by higher concentrations of nitrogen and phosphorus and an increased turbidity and stream conductivity of harvested watersheds [15,16]. On the other hand, several studies focusing on benthic algae compositions as an indicator for stream-water conditions demonstrated that ion concentrations exerted an important control on the composition of benthic communities [19,20].

From the ecological and socioeconomic points of view, the expansion of planted forests has been questioned due to its impact on biodiversity [21]. Understanding the relationship between benthic algae and forest cover factors, as well as their temporal evolution is therefore crucial in developing integrated biodiversity management policies in intensive managed plantations. The present study focuses on the relationships between forest cover, riparian vegetation, and streams to determine benthic algae composition and their variation under different forest cover schemes and conditions in the watershed headwaters. The main aim was to investigate whether physicochemical parameters or forest cover caused significant changes in algae abundance and community composition.

We hypothesized that physicochemical parameters, i.e., pH, stream conductivity, nutrient concentrations, light availability and flow velocity, caused by forest cover led to changes in algal abundance and community composition. Specifically, we predicted that forest cover would change community composition, at higher forest plantation volume, density and age, or decrease algal abundance due to canopy openness limiting light. Alternatively, we studied the potential seasonal effect in the abundance of benthic algae.

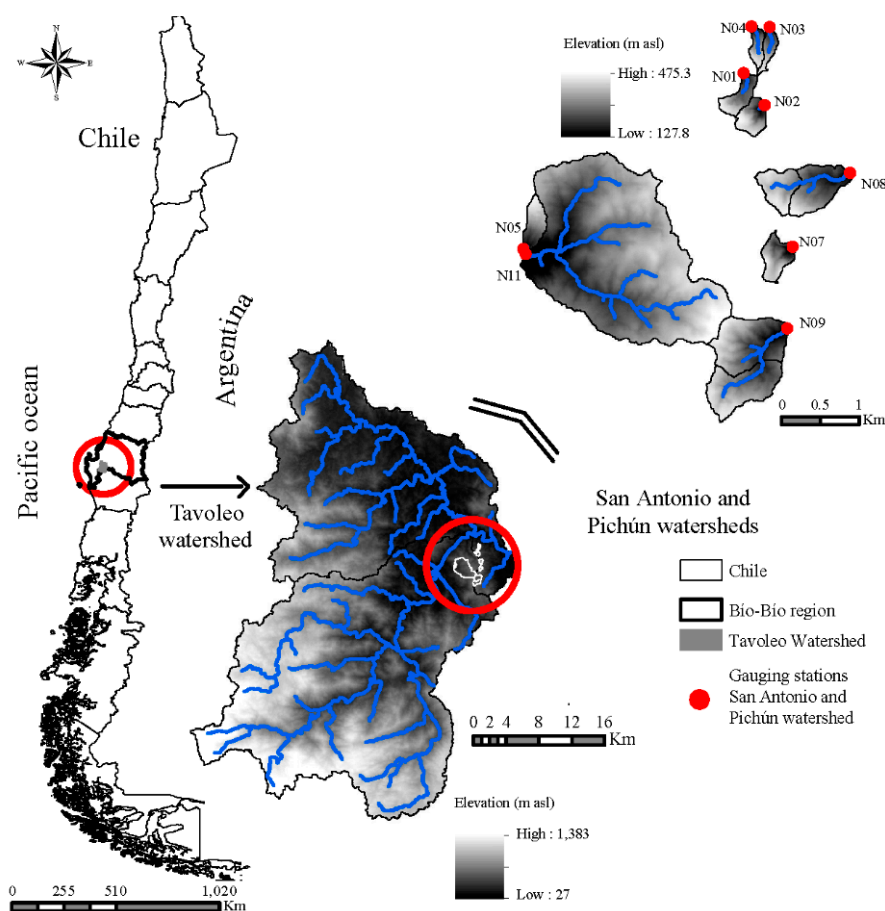
## 2. Materials and Methods

### 2.1. Study Area

The study was conducted in nine watersheds located in the Chilean Coastal Range in the Biobío Region (Figure 1) which corresponds to the central hydroclimatic zone of Chile.

The study watersheds are located 3 km west of the town of Nacimiento (37°28' S, 72°42' W) and are part of the higher-order San Antonio and Pichún watersheds. The hydrology and climate of the watersheds have been monitored since 2008. Past hydrologic investigations at Pichún and San Antonio focused on the effects of forest cover activities on water yield [22–25], peak flows [26], stream flow responses to earthquakes [27] and the accumulation of in-stream wood in low-order forested streams [28]. Detailed site descriptions of the higher- and lower-order watersheds in this hydrologic system are given by [22,27].

The study watersheds are characterized by a subtropical Mediterranean climate with dry summers. Mean long-term annual rainfall is estimated to be 1381 mm, most of which (95%) occurs between April and September during frequent and prolonged low- to moderate-intensity frontal storms. The long-term rainfall record is marked by inter-annual variations, and its spatial distribution is orographically controlled by the topography of the coastal mountain range, with elevations of up to 1383 m.a.s.l., mean slopes from 14 to 22° but exceeding 60° along the channel networks and road cuts, promoting active gully formation and erosion [27]. During rainfall events, the plantations in the study watersheds are generally immersed in mist or clouds due to their relatively low altitude (233–389 m.a.s.l.). On average, 25.5% (16.3 to 41.3%) of the annual rainfall turns into highly responsive runoff.



**Figure 1.** Sampling since 10 November 2015, and 18 August 2016 of benthic algae communities in nine study watersheds, located in San Antonio and Pichún higher-order watersheds, Chilean Coastal Range, Biobío region.

The Mediterranean climate has wet, mild winters and exceptionally dry, warm to hot summers [29]. The temperature ranges from less than  $-3^{\circ}\text{C}$  (winter) to more than  $40^{\circ}\text{C}$  (summer), with an annual average of  $13^{\circ}\text{C}$  [11,27]. The physiographic characteristics of all watersheds are summarized in Table 1. The watersheds are characterized by granitic bedrock covered by clayey to loamy soils and have areas of between  $0.07$  and  $4.1\text{ km}^2$ . The average slope is between  $27$  and  $44\%$  over an elevation range of  $233$  to  $368\text{ m.a.s.l.}$  The drainage density ranges from  $2.2$  to  $7.7\text{ km/km}^2$ .

The watersheds are mainly covered by plantation forests of the genera *Eucalyptus* and *Pinus* (Table 1). The vegetation cover varies from  $57.5$  to  $92.7\%$  for planted forests and from  $4.1$  to  $40.9\%$  for natural forests. The fluvial channels in all watersheds are bounded by a  $15$ – $70\text{ m}$  wide riparian zone with native forests and some *Pinus radiata* specimens that have invaded the area by natural regeneration during previous rotations [30,31].

The soil exhibits a thin herbaceous cover ( $<25\%$ ) mainly composed of grasses that usually perish during the summer because of water deficit [22]. The shrub cover under the most developed plantations is rather dispersed and mostly composed of the genera *Aristotelia* L'Her and *Rubus* Schott, as well as some arboreal genera such as *Luma apiculata* Burret, *Peumus boldus* Molina, *Persea lingue* Miers ex Bertero Nees and *Nothofagus dombeyi* Mirb.

The watersheds soil type is a clayey to loamy Luvisol with a variable structure on a small scale due to fragments of bedrock within the topsoil, which is a complex distribution of recent and former root systems (Table 1) [27]. Truncated soil profiles exposing low conductive B-horizons and evidence of recent incision indicate active soil erosion and landsliding, and disturbance by timber harvest, e.g., [32].

**Table 1.** Physicochemical and forest cover parameters of studied watersheds.

Parameter/Watersheds	N01	N02	N03	N04	N05	N07	N08	N09	N11
Soil type	Clayey to loamy	Clayey to loamy	Clayey to loamy	Clayey to loamy	Clayey to loamy	Loamy to clayey	Clayey to loamy	Loamy to clayey	Clayey to loamy
Geology	Granite-basalt	Granite-basalt	Granite-basalt	Granite-basalt	Granite-basalt	Quartzite- schist	Granite-basalt	Quartzite- schist	Granite-basalt
Area (km <sup>2</sup> )	0.13	0.14	0.07	0.08	0.15	0.17	0.53	0.97	4.11
Slope (m/m)	0.28	0.27	0.4	0.42	0.44	0.29	0.36	0.39	0.38
Elevation (m a.s.l)	321	323	233	234	248	360	269	368	300
Physicochemical parameters									
Solar radiation (MJm <sup>-2</sup> )	31.3	26.9	30.2	27.5	25.7	27.6	28.6	26.9	29.7
Rainfall (mm/year)	1254	1254	1476	1476	1131	1258	1231	1167	1131
Runoff (mm/year)	274.4	261.3	348.3	306.3	391.2	185.7	257.6	385.5	343.3
Water temperature (°C)	12.4 (±2.4)	12.3 (±2.0)	12.5 (±3.0)	12.8 (±3.1)	11.4 (±2.4)	12.3 (±2.5)	12.4 (±2.7)	11.8 (±2.1)	11.7 (±2.8)
Total dissolved solids (mg/L)	44.5 (±2.8)	45.1 (±2.4)	81.0 (±6.1)	68.2 (±3.8)	66.6 (±3.4)	34.1 (±5.7)	46.2 (±2.1)	36.8 (±1.1)	58.5 (±6.4)
Dissolved oxygen (mg/L)	10.1 (±1.3)	10.5 (±2.0)	11.2 (±1.7)	10.1 (±1.4)	10.4 (±0.9)	9.5 (±1.6)	10.8 (±1.4)	11.1 (±1.6)	11.1 (±0.6)
pH	7.7 (±0.2)	7.4 (±0.4)	7.5 (±0.4)	7.4 (±0.2)	7.3 (±0.1)	6.9 (±0.6)	7.6 (±0.3)	7.5 (±0.2)	7.5 (±0.1)
Conductivity (µS cm <sup>-2</sup> )	44.3 (±2.6)	44.9 (±2.2)	81.0 (±6.0)	68.2 (±3.7)	66.4 (±3.5)	34.1 (±5.6)	46.1 (±1.9)	36.7 (±1.0)	58.0 (±6.4)
Nitrate (NO <sub>3</sub> -N µg/L)	49.6 (±35.8)	30.5 (±18.1)	7.5 (±2.8)	10.3 (±4.3)	11.2 (±3.5)	11.6 (±5.9)	16.8 (±7.5)	9.7 (±4.4)	12.4 (±6.2)
Nitrite (NO <sub>2</sub> -N µg/L)	<2 (±0.01)	<2 (±0.07)	<2 (±0)	<2 (±0)	<2 (±0)	<2 (±0)	<2 (±0.03)	<2 (±0)	<2 (±0)
Ammonium (NH <sub>4</sub> -N µg/L)	14.2 (±6.9)	14.5 (±7.0)	15.1 (±6.7)	15.4 (±6.7)	15.9 (±7.5)	16.5 (±8.0)	16.7 (±8.0)	17.8 (±9.9)	21.6 (±18.9)
Soluble phosphorus (PO <sub>4</sub> -P µg/L)	4.1 (±1.1)	8.2 (±2.9)	10.7 (±5.4)	3.2 (±0.8)	6.3 (±2.9)	2.1 (±0.4)	3.3 (±1.3)	3.7 (±1.1)	4.7 (±3.3)
Forest cover parameters									
Species	<i>Pinus radiata</i>	<i>Pinus radiata</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>	<i>Eucalyptus globulus</i>
Canopy Openness %	22.9	20.8	46.2	42.4	54.4	18.2	34.1	18.4	26.1
Solar radiation SRt (Mols m <sup>-2</sup> d <sup>-1</sup> )	11.8	6.4	20.8	15.1	24.3	3.17	12.6	5.1	10.3
Roads area %	2.7	3.2	2	1.9	1.6	2	1.8	1.9	3
Plantation area %	88.0	92.7	74.9	87.7	78.9	86.4	86.5	57.5	65.6
Native forest area %	6	4.1	22.7	10.5	19.5	9.3	11.7	40.4	21.1
Harvested area %	3.3	0	0.4	0	0	2.3	0	0.2	10.3
Planting volume (m <sup>3</sup> /ha)	303.2	295.1	308.8	234.9	433.4	183.9	251.9	506.8	397.4
Planting density (No. tree/ha)	232	308	1147	1070	1072	1130	529	1232	1158
Age plantation (year)	28	28	5	5	8	3	3	15	9
Width riparian zone (Wrz) (m)	16.5	15.5	35.7	17.7	40.4	23.9	21	49.7	70.5

Instream samples (Water temperature, Total dissolved solids, Dissolved oxygen, pH, Conductivity, Nitrate, Nitrite, Ammonium and Soluble phosphorus) each 45 days between 10 November 2015 and 18 August 2016. Solar radiation transmitted through openings in the Canopy (SRt). Physicochemical parameters and characterization of Forest cover parameters using methodology described in [11,31]. ± for reference standard deviation is also show.

## 2.2. Physiographic Variables and Forest Cover in the Study Watersheds

We used detailed LiDAR (1 m resolution DEM) to calculate the physiographic variables, including watershed area ( $\text{km}^2$ ), average watershed slope ( $G$  [%], the median value of slopes for all grid cells in a watershed, and elevation (m.a.s.l.) [31]. Photointerpretation of LiDAR images provided the characterization of forest use, as follows: the areas of planted and native forests and roads; harvested land; and the average width of the riparian zone (m). For each of the analyzed parameters, the different forest cover uses (plantation, native forests, roads, harvested and riparian zone) in each watershed were digitized in polygons using the vector layer method. Once the layers were digitized, the respective areas were calculated in relation to the total area of each watershed to obtain the percentage of different land uses [11,31].

The distance (m) between the vector layers corresponding to the main fluvial channel and the outer limit of the riparian zone was used to create a raster layer for calculating the average width of the riparian areas [31]. The identification of planted species was performed by image recognition and validated by field check to confirm the identified species. Finally, the age of planting (year), the planting volume ( $\text{m}^3/\text{ha}$ ), the planting density (trees/ha) and the soil type were obtained through digital coverage (format shape) support from Forestal Mininco S.A. The landscape variables are summarized in Table 1.

## 2.3. Stream Physicochemical Parameters

Stream water samples for the analysis of nitrate, nitrite, ammonium, and soluble phosphorus concentrations were collected in 500 mL bottles once every 45 days between 10 November 2015 and 18 August 2016. The samples were subjected to segmented flow analyses using a SKALAR module and standard APHA methods (2005), as follows: the 4500 E standard method (2005) was used for instream nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) and ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ), with  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  concentration with an L.D. of 0.002 mg N/L and for  $\text{NH}_4\text{-N}$  concentration with an L.D. of 0.003 mg N/L. The 4500 blue ascorbic acid method (2005) was used for soluble phosphorus ( $\text{PO}_4\text{-P}$ ) concentration with an L.D. of 0.002 mg P/L. Water temperature, dissolved oxygen, pH, conductivity, and turbidity were measured at each site using a calibrated brand WTW® model 350i multiparameter probe.

Digital hemispherical canopy photographs were taken above each algae collection point (one photo per habitat type), using a Sony Alpha 37 camera equipped with a fisheye lens. The hemispherical canopy photographs were overlain by the sun-path to calculate the total amount of solar radiation ( $\text{MJm}^{-2}$ ), the amount transmitted through openings in the canopy ( $\text{Srt}$ ) ( $\text{Mols m}^{-2} \text{ d}^{-1}$ ) and the percent openings in the canopy (%) [16,33]. According to [16,34], each photograph was processed and analyzed with a gap light analyzer (GLA). (See Table S1. Additional supporting information, Gap light analyzer (GLA) program setup).

## 2.4. Benthic Algae

Benthic algae were sampled in all nine of the studied watersheds upstream of the fluviometric control points (Thompson type concrete dumps with a  $V$  of  $60^\circ$ ) between 10 November 2015 and 18 August 2016. Non-qualitative sampling was carried out using artificial substrates of plastic paper that firmly adhered to porcelain tile blocks of  $22.1 \times 28$  cm that were standardized in color, texture, and size and immersed at the sampling sites. The block was placed at the bottom of the river, between the stones, at a depth of approximately 50 cm. The block remained at the bottom of the river for a period of 45 days. The sheet material was transferred using a soft bristle brush, and the resulting suspension was collected and preserved in bottles with 10% Lugol's solution. All fixed samples were stored, protected from light and in a cool place ( $<15^\circ\text{C}$ ). In the laboratory, a quantitative analysis of the samples that included a count of the benthic algae genera (diversity) and total individuals present of benthic algae (abundance) was carried out via subsample extraction from the field specimen. A drop of the suspension was placed



with a Pasteur pipette between a slide and coverslip to be observed under an inverted microscope with a digital camera attached [35]. We counted >1000 cells as total samples at  $\times 100$ -magnification. The taxonomic (genera) identification was conducted on the basis of the specialized bibliography in [35–38] and the specialized literature for each group from studies including [36,39–51].

### 2.5. Data Analysis

All statistical analyses were performed using the R software package [52]. Kruskal–Wallis (KW) non-parametric tests were used to derive the statistical significance of differences ( $p \leq 0.05$ ) of diversity (count of the benthic algae genera) and abundance (total individuals present).

We correlated the abundance data (total individuals present and genera categorization) with physicochemical parameters, canopy, solar radiation, and forest cover data described in Table 1 using linear regression (linear correlation coefficient ( $R$ ) and results were considered statistically significant if the  $p \leq 0.05$ ).

The analysis of similarity (ANOSIM) was used to evaluate whether there was a significant difference between two or more groups of sampling units [53]. ANOSIM provides a robust measure of magnitude of abundance change associated with each factor (physiochemical parameters, canopy, solar radiation, and forest cover characteristics), having excised any possible effect from the factor(s) it is crossed with, irrespective of whether the factors interact or not [53]. There is no replication, as there are no within-group dissimilarities. Instead, the effect of the factor (physiochemical parameters, canopy, solar radiation, and forest cover characteristics) is determined by assessing whether there is evidence of a common pattern among the different levels of abundance when examined for each of the levels of the factor [54].

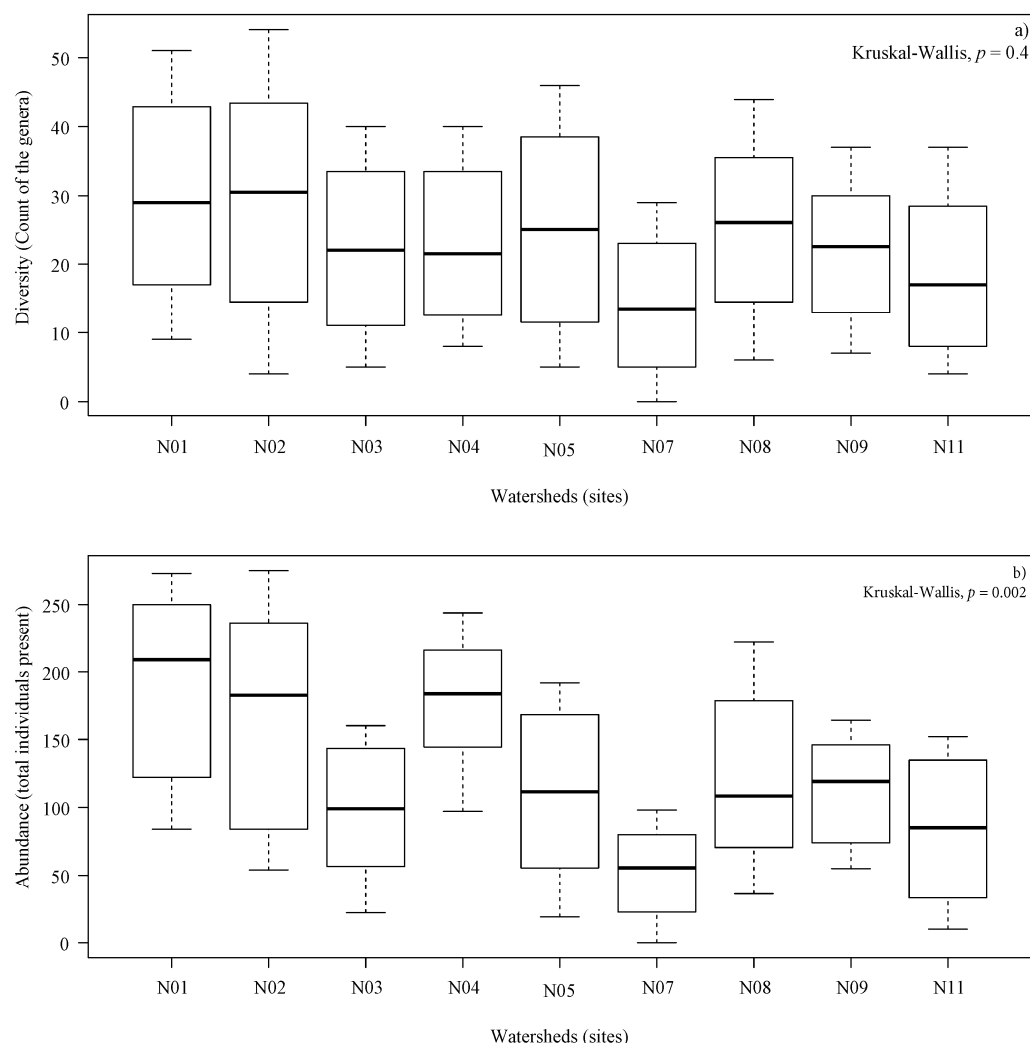
ANOSIM was calculated using Bray–Curtis distances with 999 Monte Carlo permutations [16,53], which compares the similarities of the abundance with different physiochemical parameters, canopy, solar radiation, and forest cover characteristics. Specifically, the following were examined: a box plot of rank dissimilarities ( $R$  (slope)) among the nine sites of the abundance against a) the four seasons ranks (Autumn: April and May; Winter: July and August; Spring: November and December; Summer: January and March), (b) type vegetations ranks (*Eucalyptus globulus* and *Pinus radiata*), (c) native forest area ranks, (d) plantation area ranks, (e) harvested area ranks, (f) canopy openness ranks, (g) solar radiation transmitted ranks, (h) planting volume ranks, (i) planting density ranks and (j) age of planting ranks, from a serial ordering model of groups..

## 3. Results

### 3.1. Benthic Algae

Although all sites exhibited substantial variability, the maximum diversity (count of the genera) and abundance (total individuals present) occurred on watersheds covered with *Pinus radiata*. During the first sampling at the beginning of the study, diversity ranged from 0 (N07) to 9 (N01). Diversity increased during the study, reaching a maximum of 29 cumulative genera in watershed N07 covered with *Eucalyptus globulus*, and 54 cumulative genera in watershed N02 covered with *Pinus radiata* (Figure 2a). The total abundance increased during the study, reaching a minimum of 7 and maximum of 275 cumulative individuals (Figure 2b). During the study, no significant differences in the diversity between watersheds could be estimated ( $p = 0.4$ ); however, significant differences were found in total abundance ( $p = 0.002$ ).

The resulting data matrix can be seen in Table 2. In total, 22 different genera were categorized from the nine watersheds (eight samples per watershed). The most common benthic algae were *Gomphonema* Ehrenberg, *Closterium* Brébisson, *Fragilaria* Lyngbye and *Navicula* Bory.



**Figure 2.** (a) Distribution of diversity (count of the genera) and (b) Distribution of abundance (total individuals present) in each watershed collected, 8 samples per watershed during sampling between 10 November 2015 and 18 August 2016. In (a,b),  $p$ -value using Kruskal–Wallis nonparametric test. The line within each box indicates the median value, box ends are the 25th and 75th percentiles, and the whiskers show the largest value within a 1.5 interquartile range from third quartile and the minimum value.

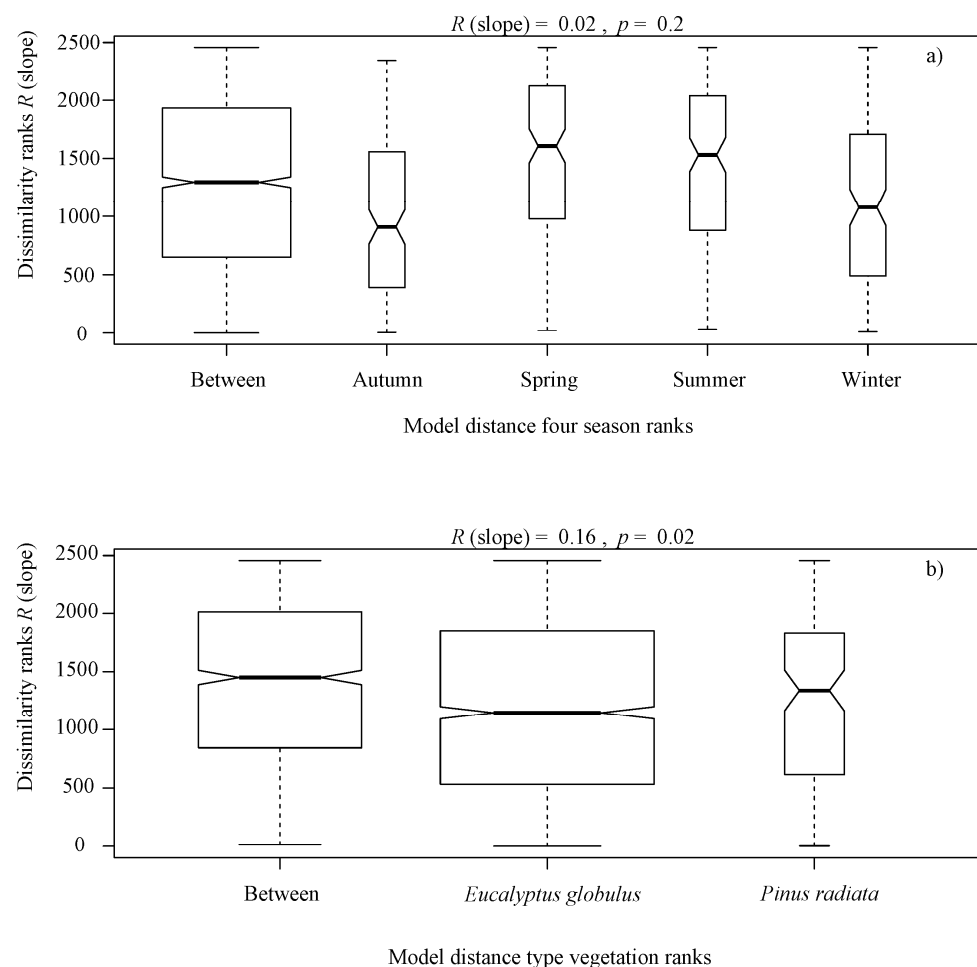
### 3.2. Anosim

The alternative hypothesis analyzed in this study provided no statistical evidence that seasonal ranks influenced the abundance of benthic algae. The ANOSIM indicated  $R$  (slope) = 0.02,  $p = 0.2$ , (Figure 3a). As there was no seasonal variation within sites, data from each study area were combined to account for the general site-specific composition of algae assemblages.

It is noteworthy that overall, the benthic algae is controlled with forest cover factors, indicating significant differences in terms of the type of vegetation ranks (*Eucalyptus globulus* and *Pinus radiata*) ( $R$  (slope) = 0.16,  $p = 0.02$ , Figure 3b), native forest area ranks ( $R$  (slope) = 0.24,  $p = 0.001$ , Figure 4a), plantation area ranks ( $R$  (slope) = 0.25,  $p = 0.001$ , Figure 4b), harvested area ranks ( $R$  (slope) = 0.18,  $p = 0.001$ , Figure 4c), canopy openness ranks ( $R$  (slope) = 0.25,  $p = 0.001$ , Figure 4d), solar radiation transmitted ranks ( $R$  (slope) = 0.24,  $p = 0.001$ , Figure 4e), planting volume ranks ( $R$  (slope) = 0.25,  $p = 0.001$ , Figure 4f), planting density ranks ( $R$  (slope) = 0.24,  $p = 0.001$ , Figure 4g) and age of planting ranks ( $R$  (slope) = 0.15,  $p = 0.001$ , Figure 4h), from a serial ordering model of groups.

**Table 2.** Resulting data matrix in total there were 22 different genera categorized from the 9 watersheds.

Genera (Cell/mL)	Watershed								
	N1	N2	N3	N4	N5	N7	N8	N9	N11
<i>Achnantes</i> Agardh	19	8	4	33	22	0	29	11	0
<i>Closterium</i> Brébisson	12	20	14	15	34	18	32	12	11
<i>Cocconeis</i> Ehrenberg	0	11	10	27	8	3	0	0	0
<i>Cosmarium</i> Corda ex Ralfs	0	16	0	0	0	0	0	0	0
<i>Cyclotella</i> Brébisson	0	25	18	0	0	0	0	0	0
<i>Cymbella</i> Ehrenberg	0	0	8	0	0	19	0	0	0
<i>Diatoma</i> Bory	0	2	15	0	0	0	0	37	0
<i>Diploneis</i> Ehrenberg ex Cleve	26	12	17	0	0	0	0	0	0
<i>Dolichospermum</i> (Ralfs ex Bornet and Flahault) P. Wacklin, L. Hoffmann and J. Komárek	3	25	3	0	24	2	0	0	5
<i>Eunotia</i> Ehrenberg	36	20	0	23	0	0	9	40	0
<i>Fragilaria</i> Lyngbye	38	5	4	16	14	20	11	15	22
<i>Gomphonema</i> Ehrenberg	15	56	18	58	18	13	24	18	11
<i>Gyrosigma</i> Hassall	0	0	0	0	31	0	0	4	0
<i>Hannaea</i> Ehrenberg	2	0	0	0	4	0	0	0	53
<i>Mastogloia</i> Thwaites ex W.Smith	11	0	4	0	0	0	0	0	0
<i>Melosira</i> Agardh	18	7	13	0	1	0	55	0	0
<i>Navicula</i> Bory	11	19	21	23	13	12	15	0	30
<i>Nitzschia</i> Hassall	0	0	0	24	7	0	25	15	7
<i>Pinnularia</i> Ehrenberg	13	0	5	25	0	0	0	0	0
<i>Pseudanabaena</i> Lauterborn	0	37	0	0	16	0	0	0	0
<i>Rhoicosphenia</i> Grunow	47	0	6	0	0	11	22	12	0
<i>Stauroneis</i> Ehrenberg	22	12	0	0	0	0	0	0	13
Abundance	273	275	160	244	192	98	222	164	152
Diversity	14	15	15	9	12	8	9	9	8

**Figure 3.** Box plot of rank dissimilarities ( $R(\text{slope})$ ) among the 9 sites against the four seasons ranks (Autumn, Winter, Spring and Summer) (a), and type vegetation ranks (*Eucalyptus globulus* and *Pinus radiata*) (b), from a serial ordering model of groups.



A relationship between the abundance and water-quality ranks was not found (i.e., abundance response between watersheds is highly variable), initially suggesting that, in general, the observed abundance may be mostly related to land use/cover rather than to changes in water quality.

### 3.3. Linear Regression on Benthic Algae Assemblage

The linear regression shows a non-significant negative slope relation between ammonium ( $R = -0.56$ ,  $p = 0.1$ ), native forest area ( $R = -0.5$ ,  $p = 0.1$ ), harvested area ( $R = -0.29$ ,  $p = 0.4$ ), planting volume ( $R = -0.11$ ,  $p = 0.7$ ), planting density ( $R = -0.79$ ,  $p = 0.01$ ) and width of riparian zone ( $R = -0.57$ ,  $p = 0.1$ ) (Figure 5). However, this linear slope specifically corresponds to a reduction in the abundance of the genera *Gyrosigma* Hassall, *Nitzschia* Hassall, *Cosmarium* Corda ex Ralfs and *Melosira* Agardh, and a positive slope with pH ( $R = 0.59$ ,  $p = 0.1$ ), nitrate ( $R = 0.69$ ,  $p = 0.04$ ), nitrite ( $R = 0.63$ ,  $p = 0.5$ ), plantation area ( $R = 0.54$ ,  $p = 0.1$ ), fraction of road's area ( $R = 0.32$ ,  $p = 0.3$ ) and planting age ( $R = 0.64$ ,  $p = 0.06$ ).

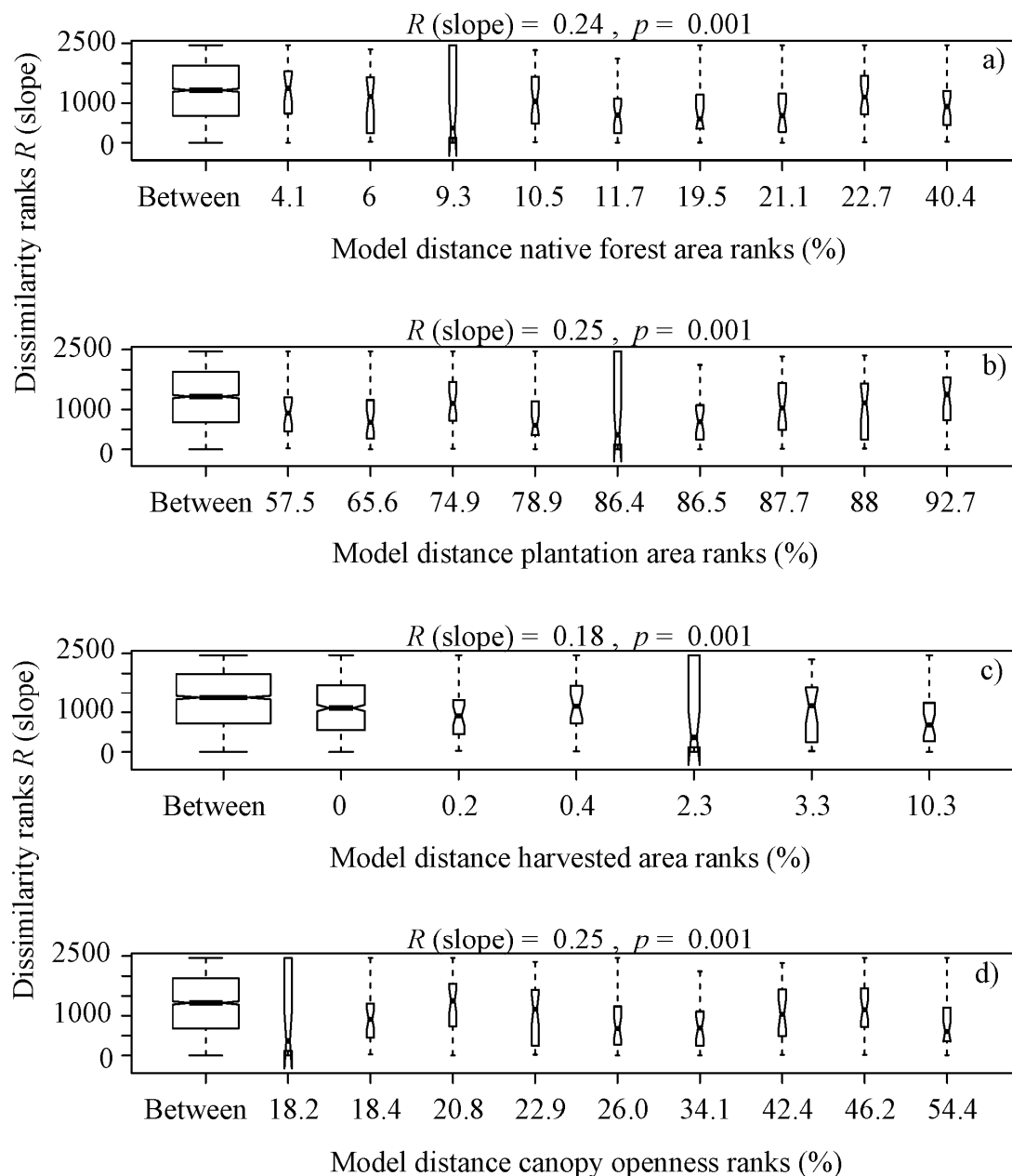
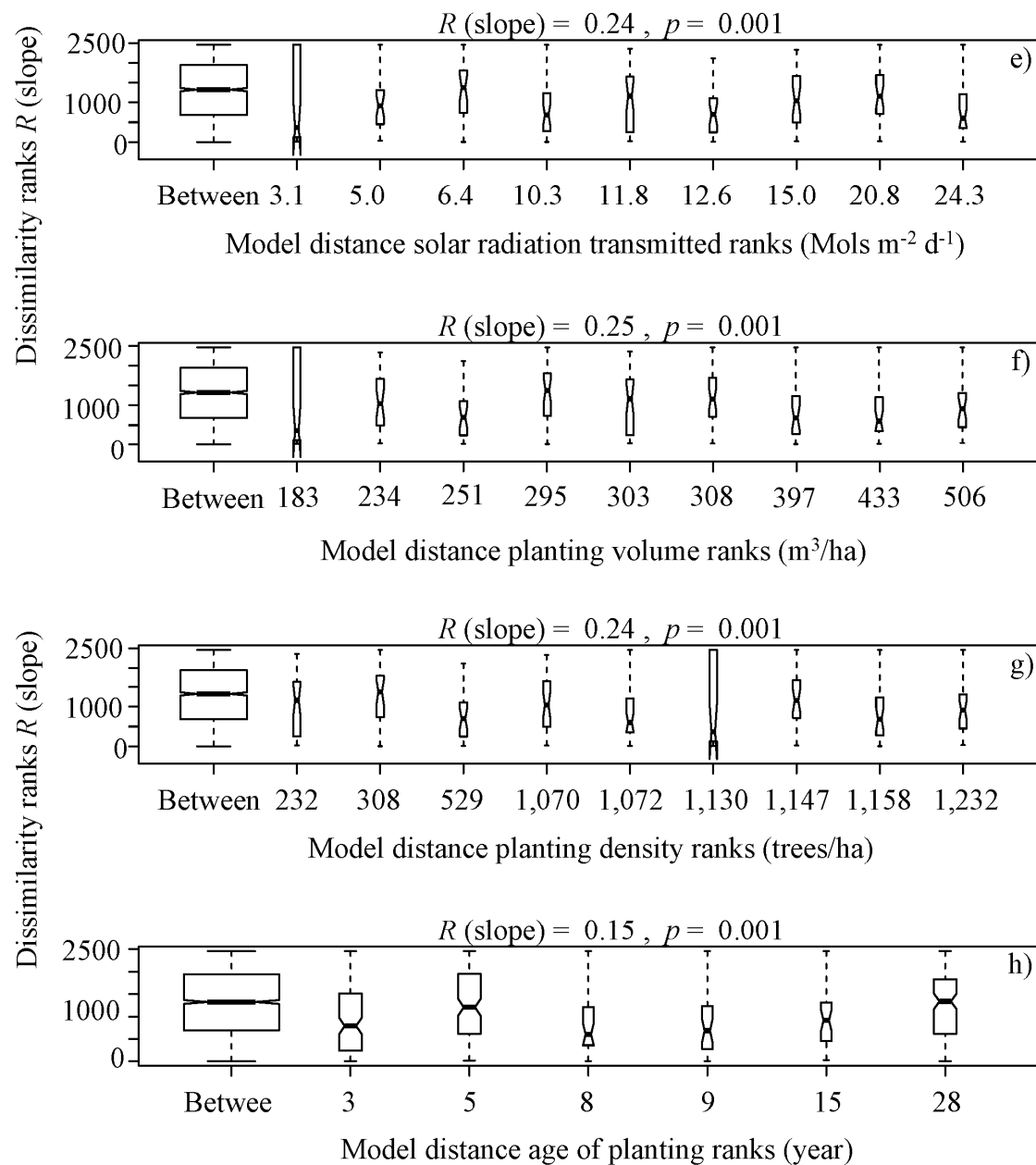


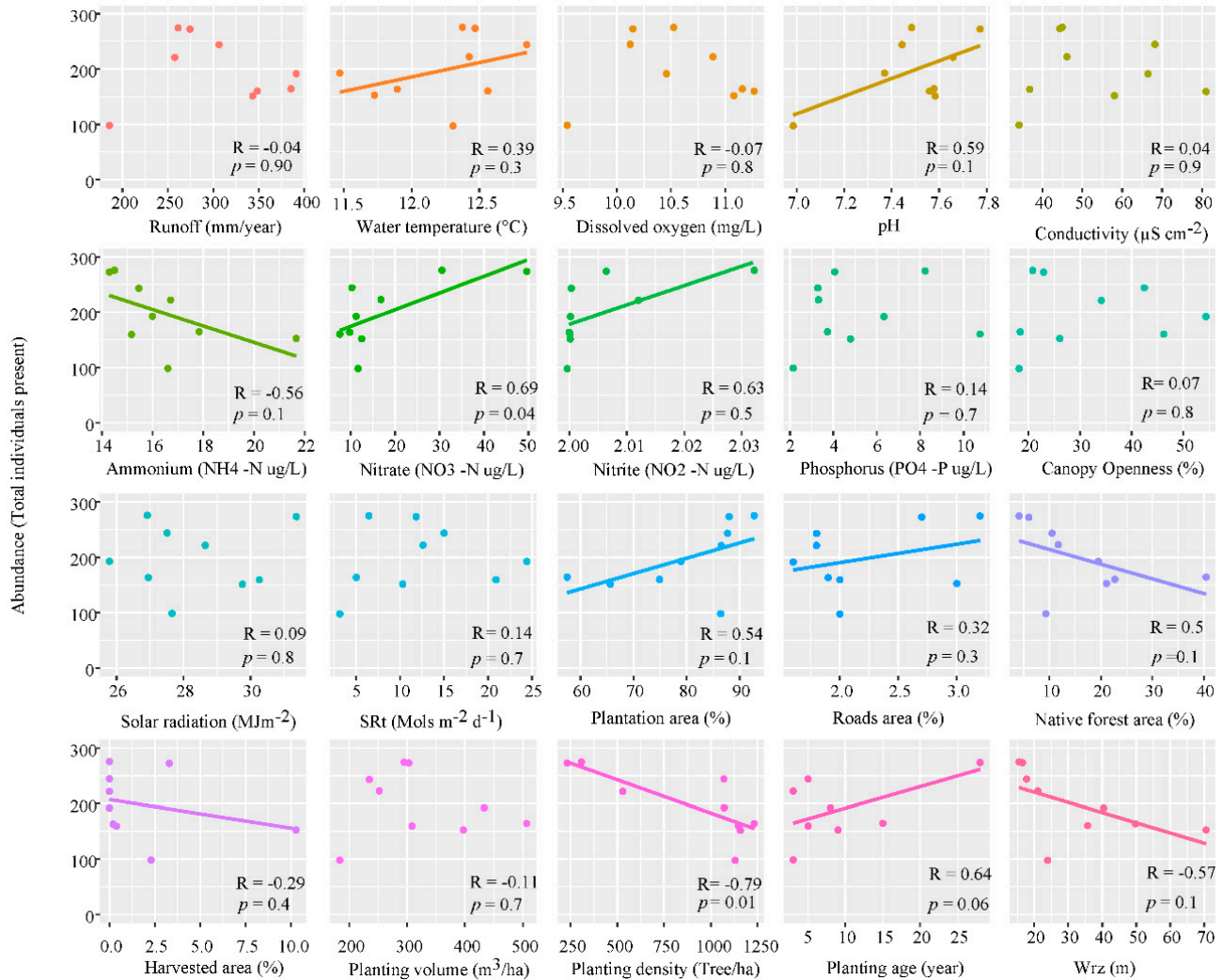
Figure 4. Cont.



**Figure 4.** Box plot of rank dissimilarities ( $R$  (slope)) among the 9 sites against native forest area ranks (a), plantation area ranks (b), harvested area ranks (c), canopy openness ranks (d), solar radiation transmitted ranks (e), planting volume ranks (f), planting density ranks (g) and age of planting ranks (h), from a serial ordering model of groups.

The nine sampling (described in Table 3) stations show a significant negative slope in the abundance of *Cymbella* Ehrenberg with increasing pH ( $R = -0.74, p = 0.02$ ), *Diatoma* Bory with plantation area ( $R = -0.73, p = 0.02$ ), *Diploneis* Ehrenberg ex Cleve with planting density ( $R = -0.73, p = 0.03$ ), *Gyrosigma* Hassall with water temperature ( $R = -0.66, p = 0.05$ ), and *Mastogloia* Thwaites ex W. Smith with increasing planting density ( $R = -0.78, p = 0.01$ ). A significant positive slope was observed in the abundance of *Cyclotella* Brébisson with phosphorus concentration ( $R = 0.86, p = 0.006$ ); *Diatoma* Bory with increasing native forest area ( $R = 0.86, p = 0.003$ ); *Diploneis* Ehrenberg ex Cleve with age of planting ( $R = 0.86, p = 0.003$ ), planting volume ( $R = 0.85, p = 0.004$ ), nitrate ( $R = 0.75, p = 0.02$ ) and solar radiation ( $R = 0.67, p = 0.05$ ); *Hannaea* Ehrenberg with ammonium concentration ( $R = 0.86, p = 0.003$ ), fractions of harvested area ( $R = 0.94, p = 0.002$ ) and width of the riparian zone ( $R = 0.78, p = 0.01$ ); *Mastogloia* Thwaites ex W. Smith with age of planting ( $R = 0.82, p = 0.01$ ),

planting volume ( $R = 0.79$ ,  $p = 0.01$ ), nitrate ( $R = 0.75$ ,  $p = 0.02$ ) and solar radiation ( $R = 0.76$ ,  $p = 0.02$ ); *Pinnularia* Ehrenberg with water temperature ( $R = 0.68$ ,  $p = 0.05$ ); *Rhoicospheria* Grunow with nitrate concentration ( $R = 0.73$ ,  $p = 0.02$ ); and *Stauroneis* Ehrenberg with nitrate ( $R = 0.86$ ,  $p = 0.003$ ) and the fraction of roads ( $R = 0.83$ ,  $p = 0.01$ ).



**Figure 5.** Linear regression of abundance, at the 9 sampling stations, against the physicochemical parameters; runoff (mm/year), water temperature (°C), dissolved oxygen (mg/L), pH, conductivity ( $\mu\text{S cm}^{-2}$ ), ammonium ( $\text{NH}_4\text{-N}$   $\mu\text{g/L}$ ), nitrate ( $\text{NO}_3\text{-N}$   $\mu\text{g/L}$ ), nitrite ( $\text{NO}_2\text{-N}$   $\mu\text{g/L}$ ), soluble phosphorus ( $\text{PO}_4\text{-P}$   $\mu\text{g/L}$ ), solar radiation ( $\text{MJm}^{-2}$ ) and solar radiation transmitted (SRT) ( $\text{Mols m}^{-2} \text{d}^{-1}$ ), and land cover data; canopy openness (%), plantation area (%), fractions of roads area (%), native forest area (%), harvested area (%), planting volume ( $\text{m}^3/\text{ha}$ ), plantation density (tree/ha), age plantation (year) and width riparian zone (Wrz) (m),  $p$ -value  $\leq 0.05$ .

**Table 3.** Linear regression of genera described in Table 2, at the 9 sampling stations against the physicochemical parameters and forest cover data, linear correlation coefficient ( $R$ ) and  $p$ -value  $\leq 0.05$ .

Parameter	Genera									
	<i>Cyclotella</i>	<i>Cymbella</i>	<i>Diatoma</i>	<i>Diploneis</i>	<i>Gyrosigma</i>	<i>Hannaea</i>	<i>Mastogloia</i>	<i>Pinnularia</i>	<i>Rhoicosphenia</i>	<i>Stauroneis</i>
	Brébisson	Ehrenberg	Bory	Ehrenberg ex Cleve	Hassall	Ehrenberg	Thwaites ex W.Smith	Ehrenberg	Grunow	Ehrenberg
Water temperature (°C)					$R = -0.66,$ $p = 0.05$			$R = 0.68,$ $p = 0.05$		
Ammonium (NH <sub>4</sub> -N µg/L)						$R = 0.86,$ $p = 0.003$				
Nitrate (NO <sub>3</sub> -N µg/L)				$R = 0.75,$ $p = 0.02$			$R = 0.75,$ $p = 0.02$		$R = 0.73,$ $p = 0.02$	$R = 0.86,$ $p = 0.003$
pH		$R = -0.74,$ $p = 0.02$								
Phosphorus (PO <sub>4</sub> -P µg/L)	$R = 0.82,$ $p = 0.006$									
Solar radiation (MJm <sup>-2</sup> )				$R = 0.67,$ $p = 0.05$			$R = 0.76,$ $p = 0.02$			
Solar radiation SRt (Mols m <sup>-2</sup> d <sup>-1</sup> )					$R = 0.42,$ $p = 0.0002$					
Native forest area (%)			$R = 0.86,$ $p = 0.003$							
Plantation area (%)			$R = -0.73,$ $p = 0.02$							
Harvested area (%)						$R = 0.94,$ $p = 0.002$				
Roads area (%)										$R = 0.83,$ $p = 0.01$
Planting volume (m <sup>3</sup> /ha)				$R = 0.85,$ $p = 0.004$			$R = 0.79,$ $p = 0.01$			
Planting density (tree/ha)				$R = -0.73,$ $p = 0.03$			$R = -0.78,$ $p = 0.01$			
Age plantation (year)				$R = 0.86,$ $p = 0.003$			$R = 0.82,$ $p = 0.01$			
Width riparian zone (Wrz) (m)						$R = 0.78,$ $p = 0.01$				

#### 4. Discussion

It proves challenging to determine whether land cover, water conditions or sunlight exert the greatest influence on the abundance of benthic algae genera. All these factors might exert a strong influence on the assemblage structure of its abundance in complex ecosystems and their ability to adapt to large-scale environmental changes. Intensive forest plantations have been found to be responsible for a series of negative effects on stream systems [23–25,28], each of which may affect abundance in different ways [5,6,19,20]. The use of herbicides to prepare the soil prior to planting and the application of pesticides to combat the pathologies that affect the plantations [55,56] also may affect abundance.

This research found that that higher percentages of forest plantation (>50%) lead to a higher diversity, characterized by facilitating the growth of benthic algae genera, which may serve as a reliable indicator of forest ecosystem quality.

To understand how biotic organisms respond to these components, we must understand the nature of the streams. A multitude of factors operate at various spatial and temporal scales [57]. Incorporating a hierarchical framework, including temporal structure and spatial heterogeneity may strongly influence the assemblage structure across an understanding of complex ecosystems and the ability to deal with large-scale environmental changes.

Our study is the first in the Chilean Coastal Range to compare the effect of forest management on the structural and functional attributes of small streams. Final harvest removed the forest cover, and associated soil erosion and changes in hydromorphology led to a major reorganization of algae communities (*Pseudanbaena*, *cyanobacteria*) [5,6,19,20].

The influence of total light transmitted through the canopy is associated with the cover type, which can influence the light levels received by the benthic algae in the stream. Dense forest covers reduce the amount of incoming energy and nutrients, shifting the abundance of algal groups [18]. We observed that when canopy openness was higher than 25%, the abundance of benthic algae was greater (positively correlation with *Diploneis* Ehrenberg ex Cleve, *Mastogloia* Thwaites ex W. Smith and *Gyrosigma* Hassall), but when canopy openness was less than 25%, benthic algae was less abundant, which is associated with less light. The influence of watershed-scale disturbances such as timber harvest was correlated with community changes, which were explained by the total light transmitted through the canopy and associated with the cover type as compared to unharvested watersheds [15].

Nutrient availability (soluble phosphorus, ammonium, and nitrate) is most strongly correlated with the abundance of the *Hannaea* Ehrenberg, *Diploneis* Ehrenberg ex Cleve, *Cyclotella* Brébisson, *Rhoicosphenia* Grunow, and *Stauroneis* Ehrenberg [58,59]. In general, study sites are low-nutrient systems, and we found that many genera detected in this system are often associated with nutrient-poor waters, i.e., *Cocconeis* Ehrenberg, *Achnantes* Agardh and *Diatoma* Bory [16,60].

These results may be used in future studies to predict the available energy for primary consumers and trade-offs between gross primary productivity and community assemblage [16]. By viewing our stream community as a system organized and developed around forest management [21,61,62], stream communities can be viewed as systems that are organized within this habitat. While the uniqueness of the sites is a general feature of these watersheds, and since forestry practices are tailored for each specific site, more studies are needed to describe biological communities and to facilitate the design of forest/water management strategies (i.e., increasing riparian buffer width, implementing sediment retention measures) aimed at the conservation of aquatic biota, while at the same time maintaining the productivity of planted forests.

#### 5. Conclusions

Overall, the results suggest that observed abundance is determined by forest use/cover rather than to changes in water-quality parameters. This study is relevant to support the already ongoing discussions related to forest management intended to minimize the impact of forest cover on abundance of genera. The study focused on small watersheds, as they are likely to be especially affected by land use. The use of abundance of genera demonstrated

the potential of a quantitative analysis. At sites where watersheds are planted at a rate of 50% or more, it is especially important that strategies are enacted to reduce potential damage to small watersheds, including the application of discontinuous forestry and maintenance or an increase in the width of the riparian zone.

Such activities will likely help to reduce toxicity algae in watersheds, which is of benefit to maintain water quality both for aquatic communities and downstream water management. Future work should concentrate on understanding how intensive forest plantation practices might interact with the flow pathways that contribute to ammonium, nitrite, nitrate, and phosphorus concentrations.

**Supplementary Materials:** Following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13030378/s1>, Table S1: Gap light analyzer (GLA) program setup).

**Author Contributions:** G.B. contributed to the project idea development, methodology, results and prepared the manuscript (writing the original draft, reviewing and editing, collecting and preparing the figures); A.I. supervised the research, revised-editing the manuscript and project administration; H.U. revised-editing the manuscript; S.B. and O.P. supervised the methodology of benthic algae and revised-editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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